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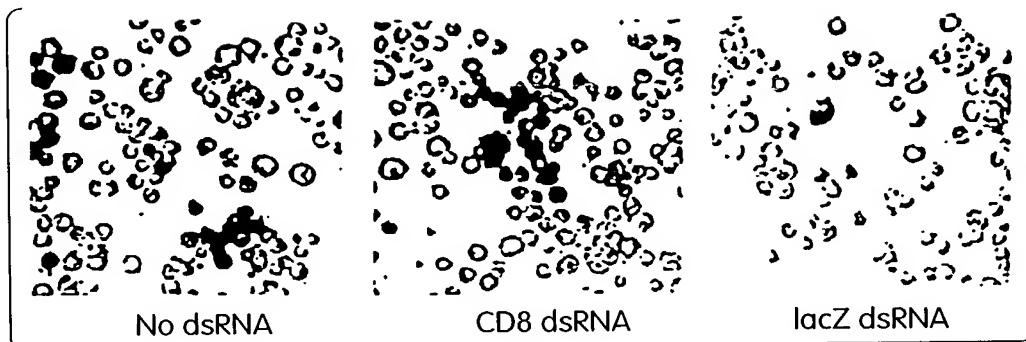


Fig. 1A

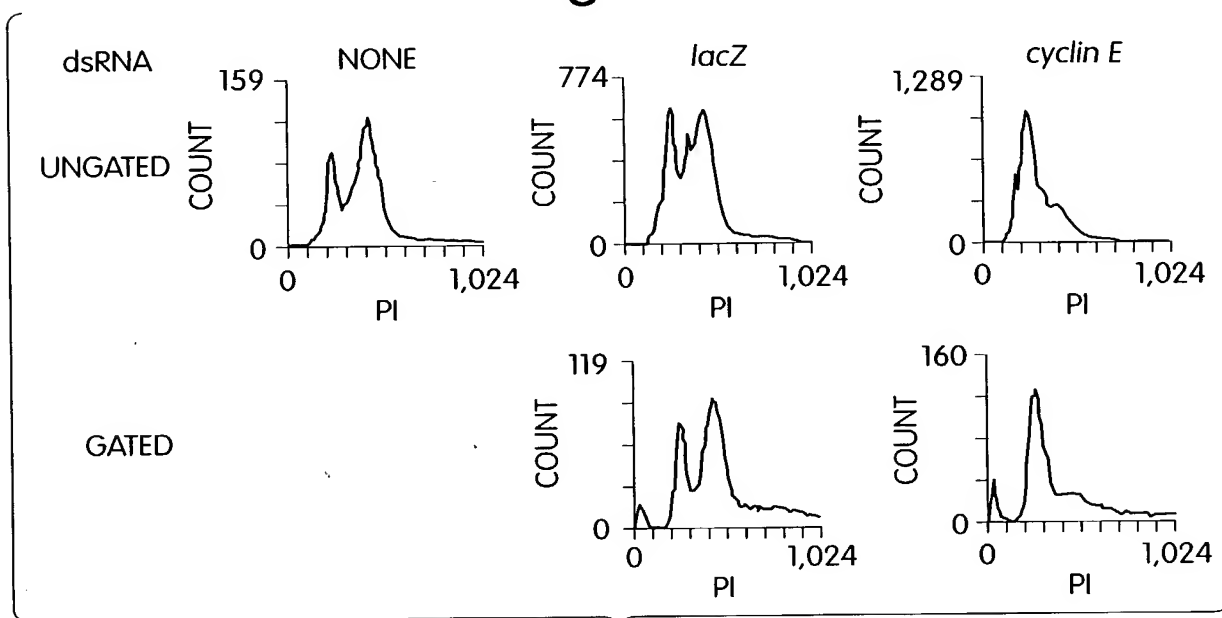


Fig. 1 B

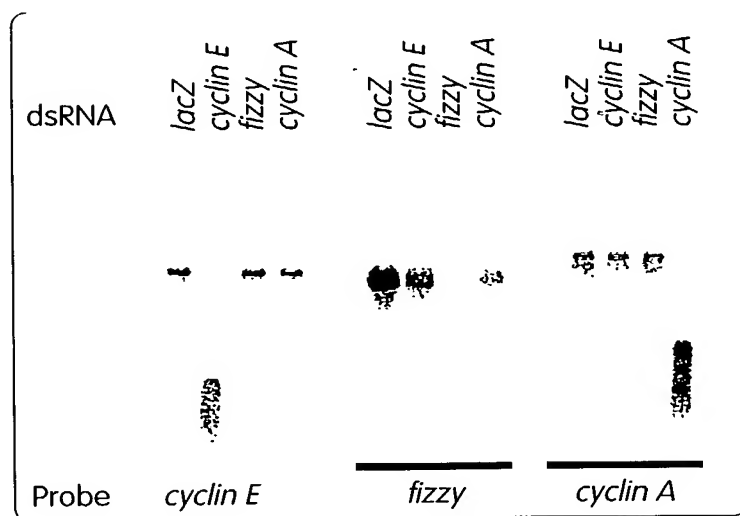


Fig. 1C

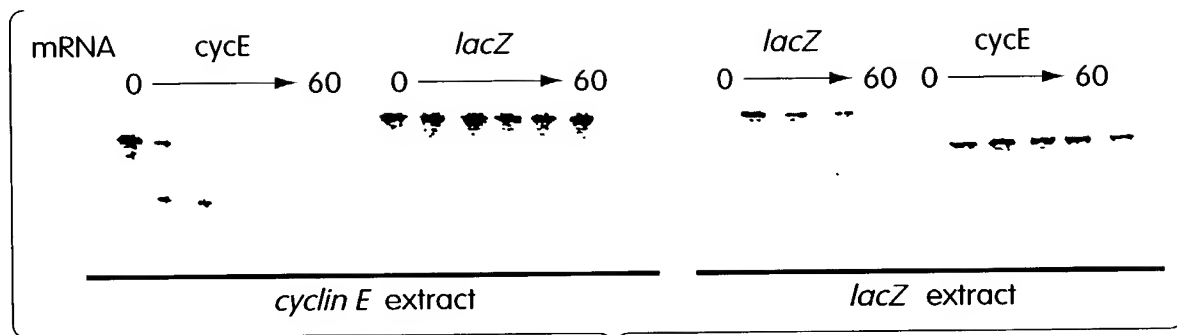


Fig. 2A

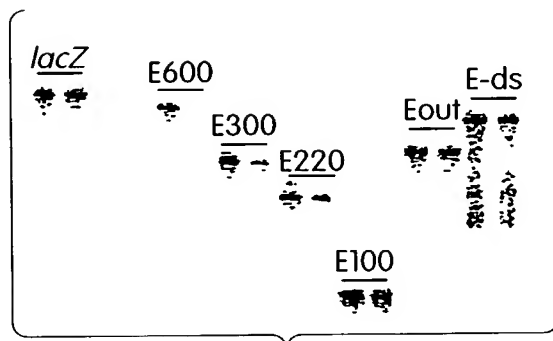


Fig. 2B

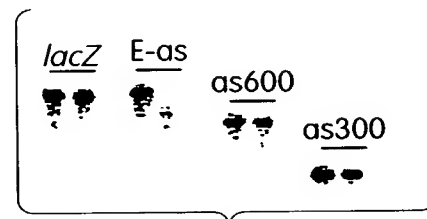


Fig. 2C

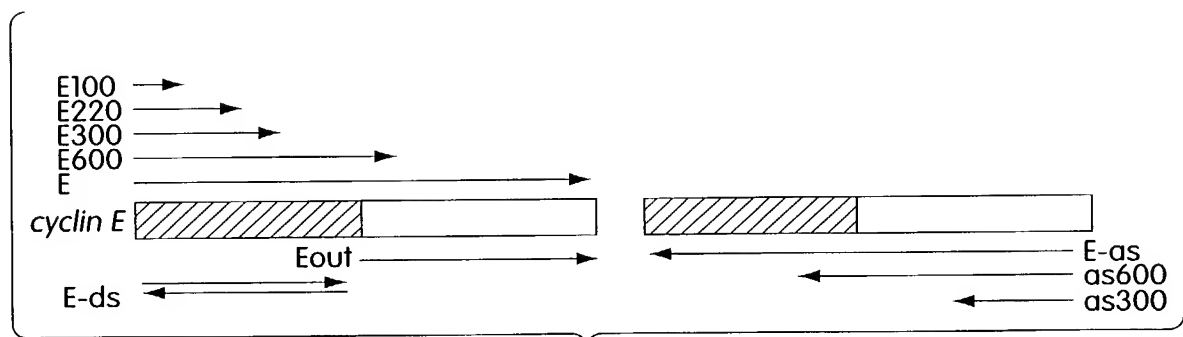


Fig. 2D

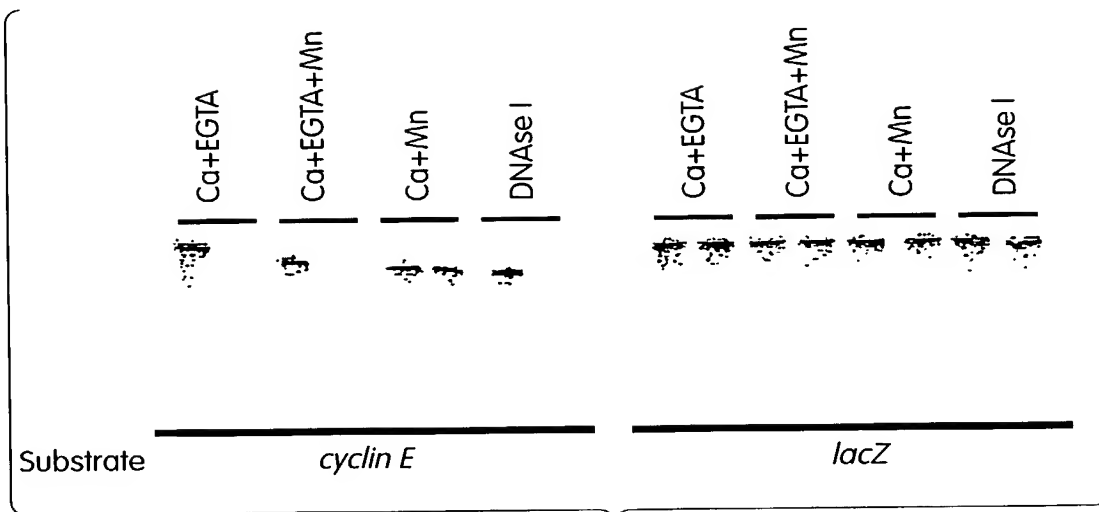


Fig. 3



Fig. 4A

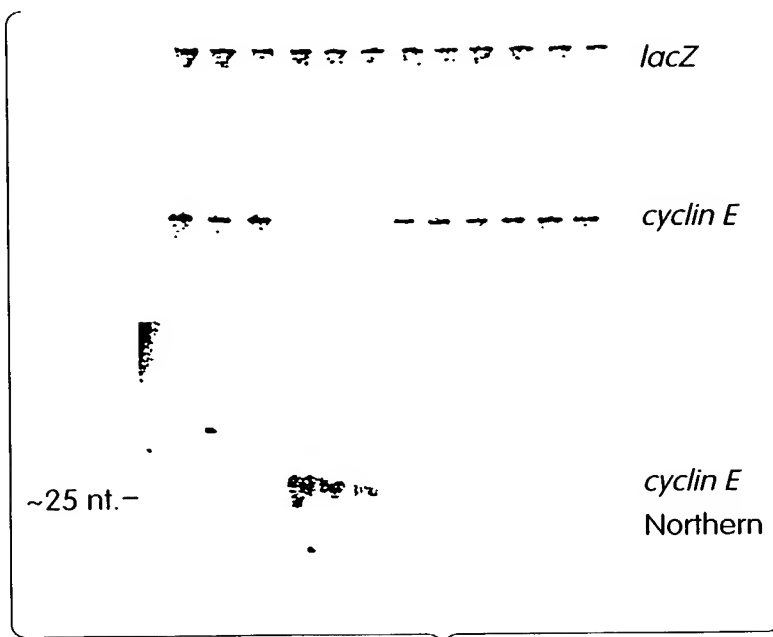


Fig. 4B

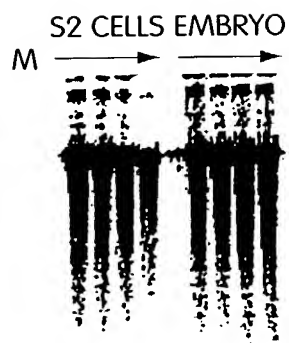


Fig. 5A

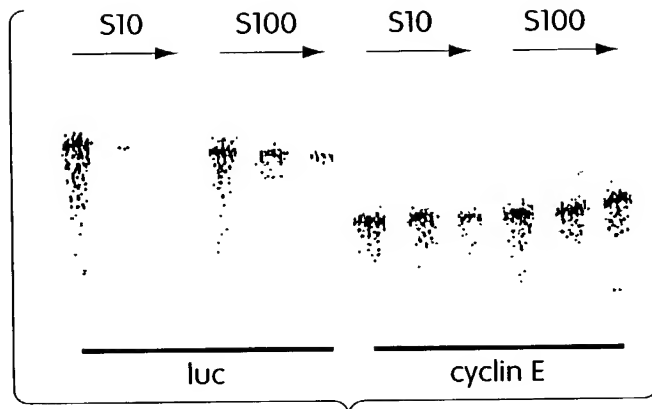


Fig. 5B

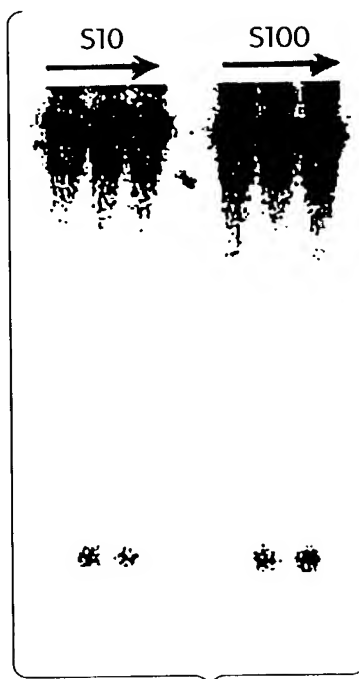


Fig. 5C

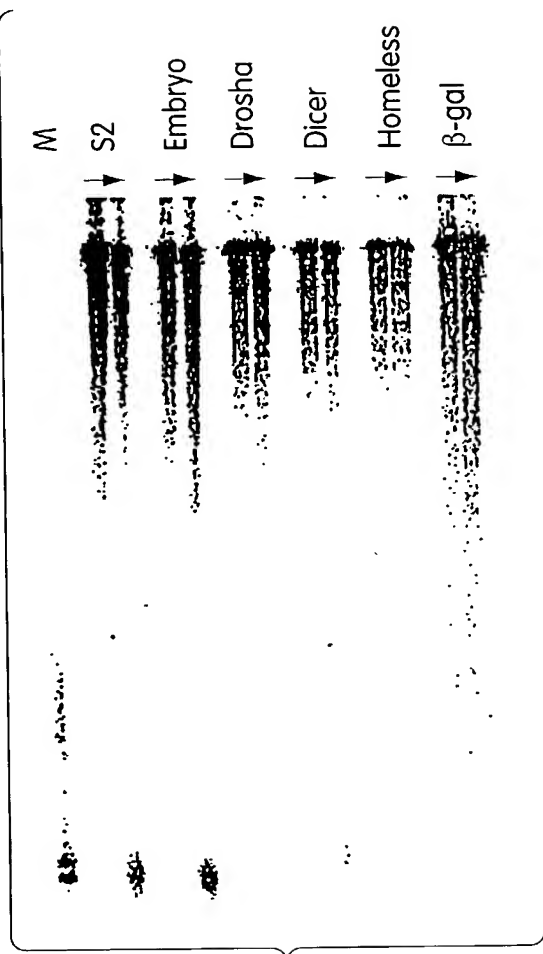


Fig. 6A

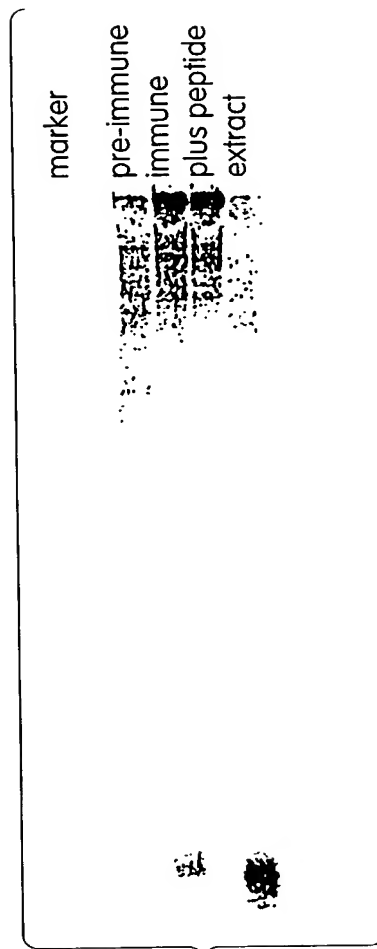


Fig. 6C

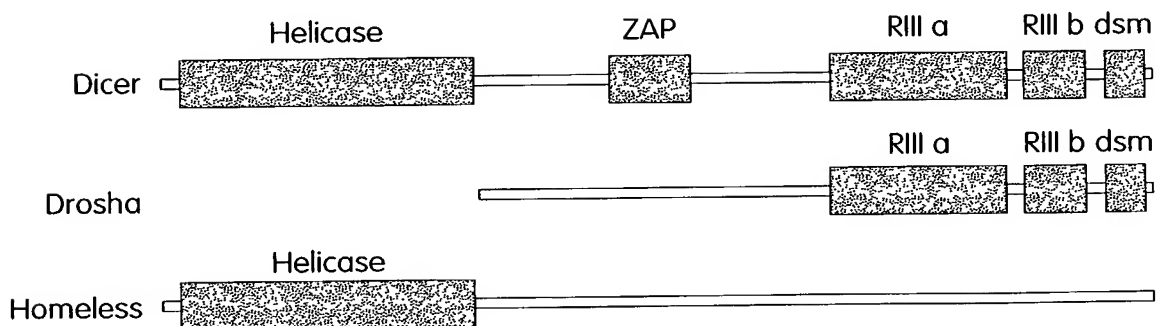


Fig. 6B



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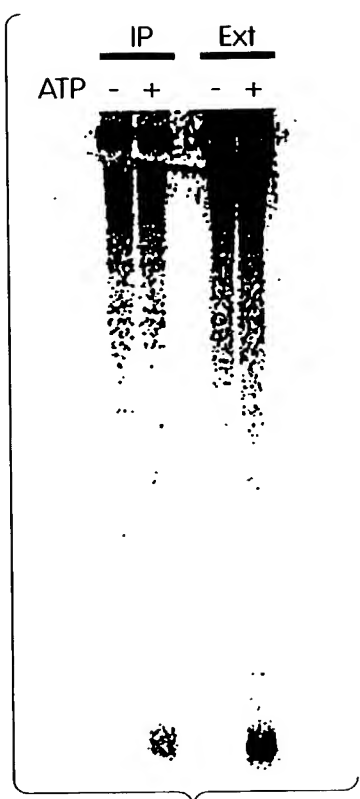


Fig. 6D

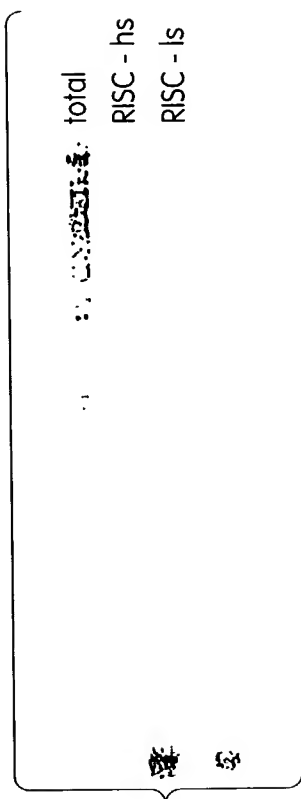


Fig. 6E

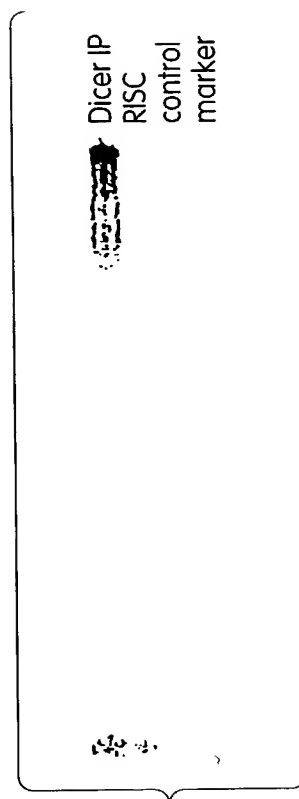


Fig. 6F



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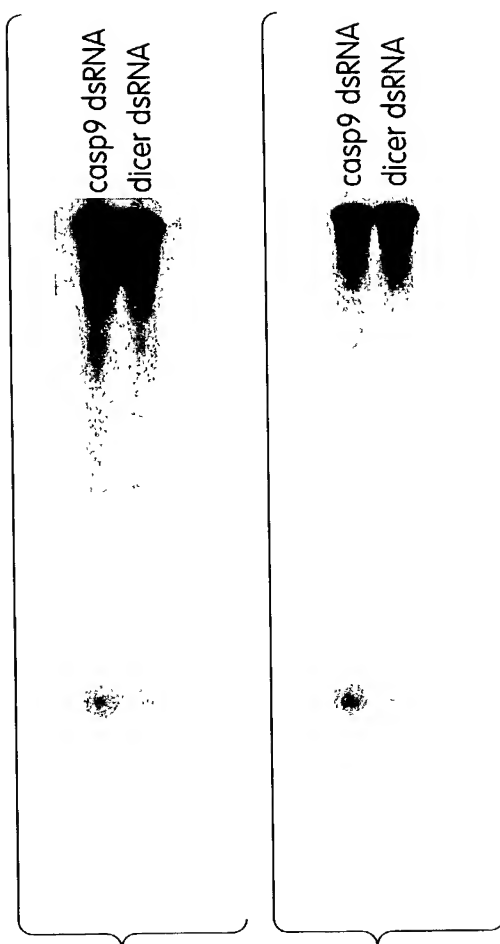


Fig. 7A

Fig. 7B

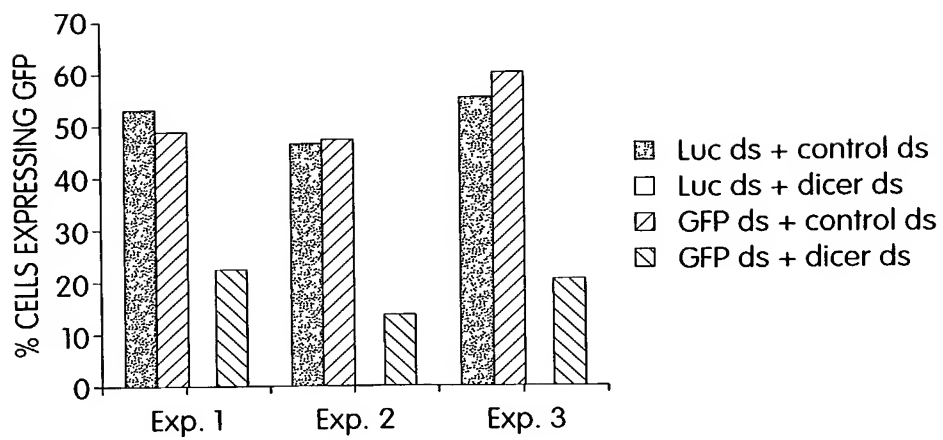


Fig. 7C



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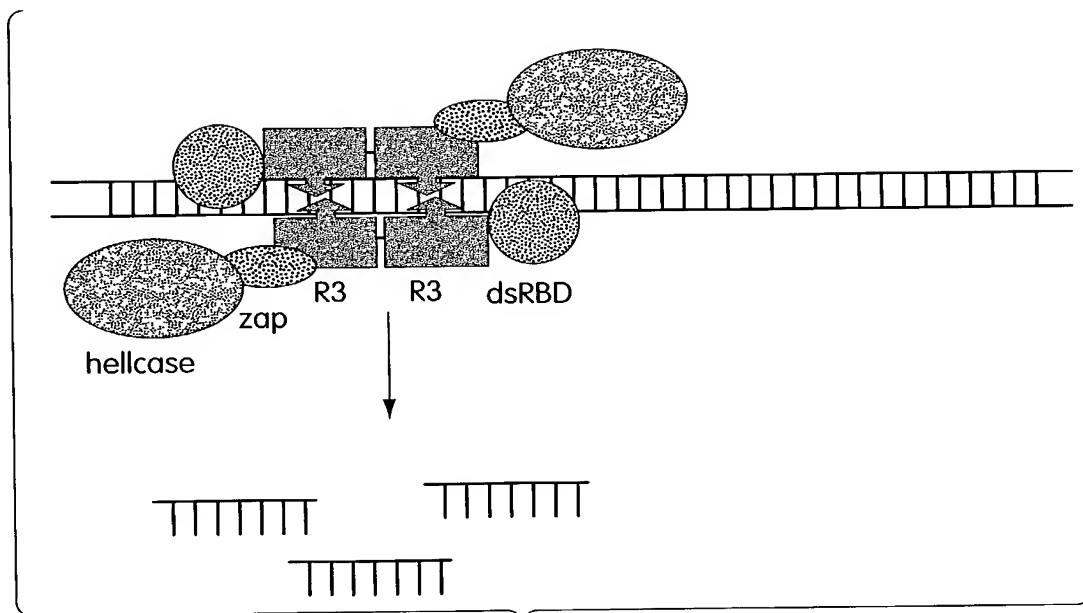


Fig. 8A

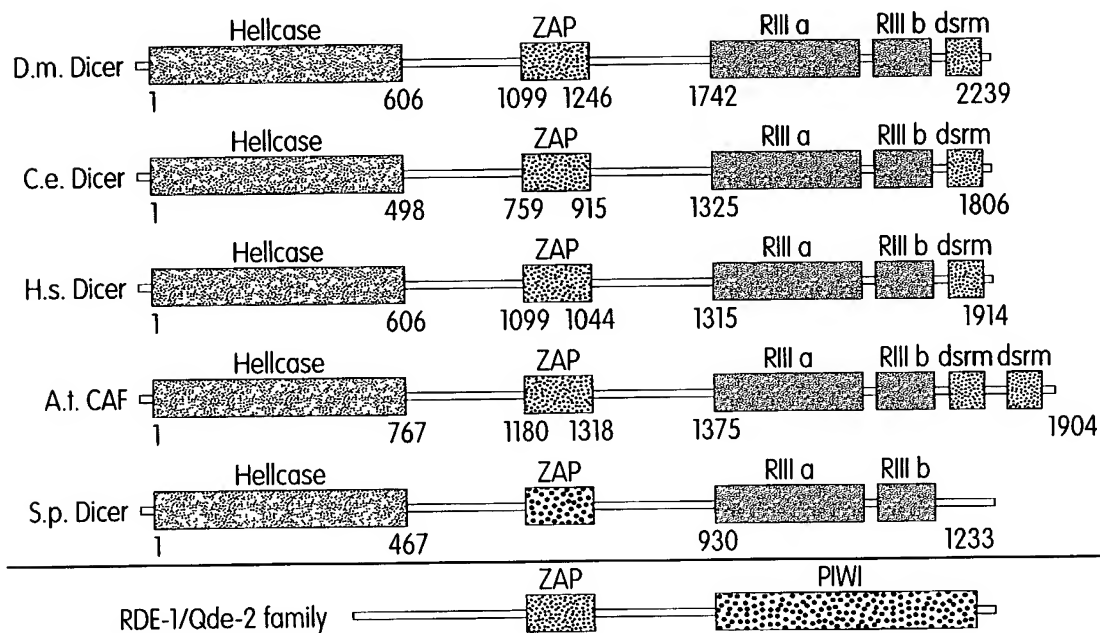


Fig. 8B

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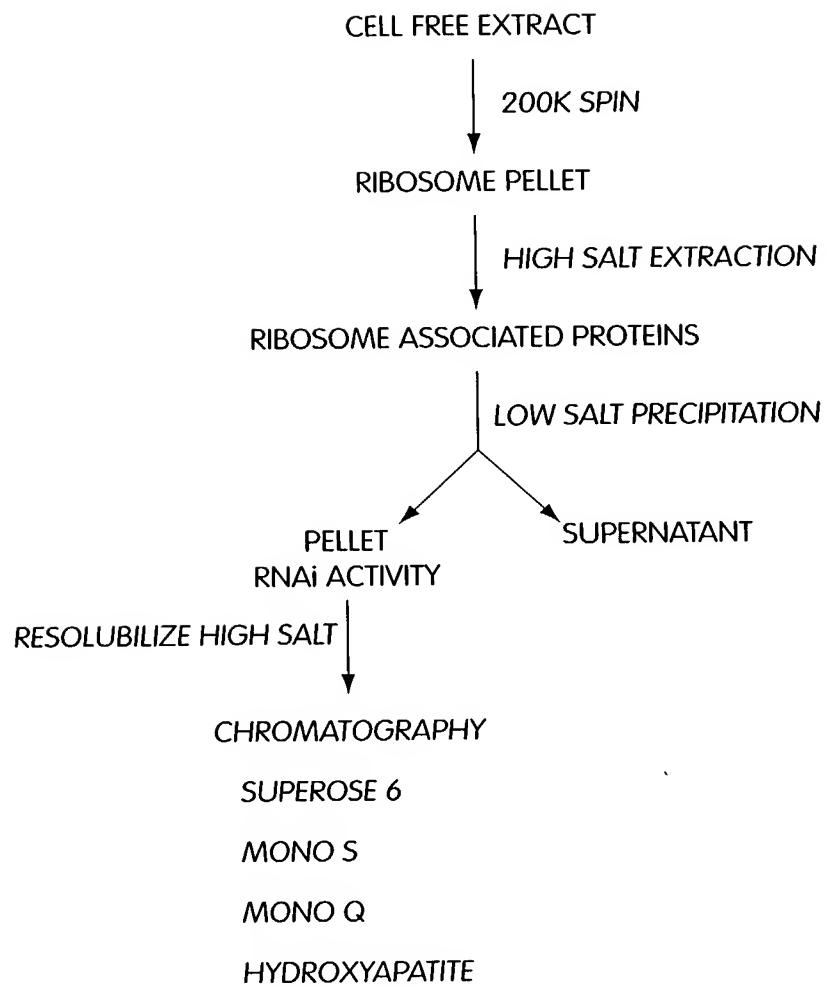


Fig. 9



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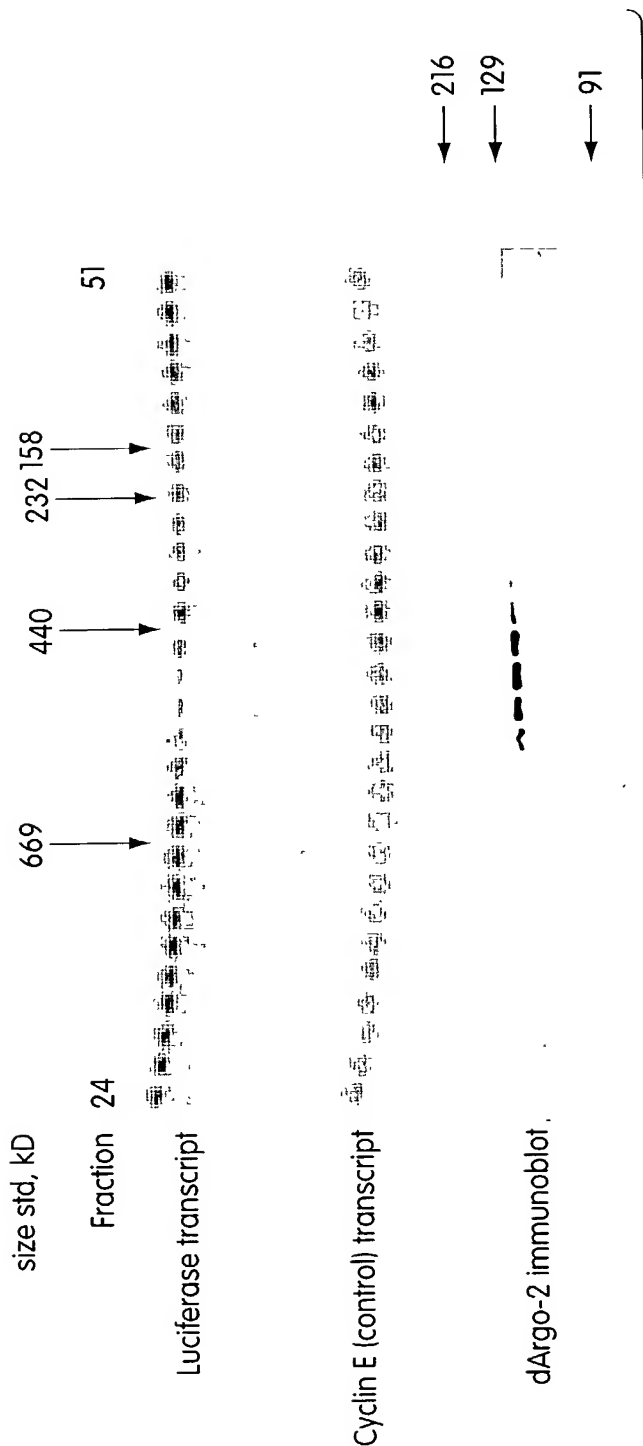
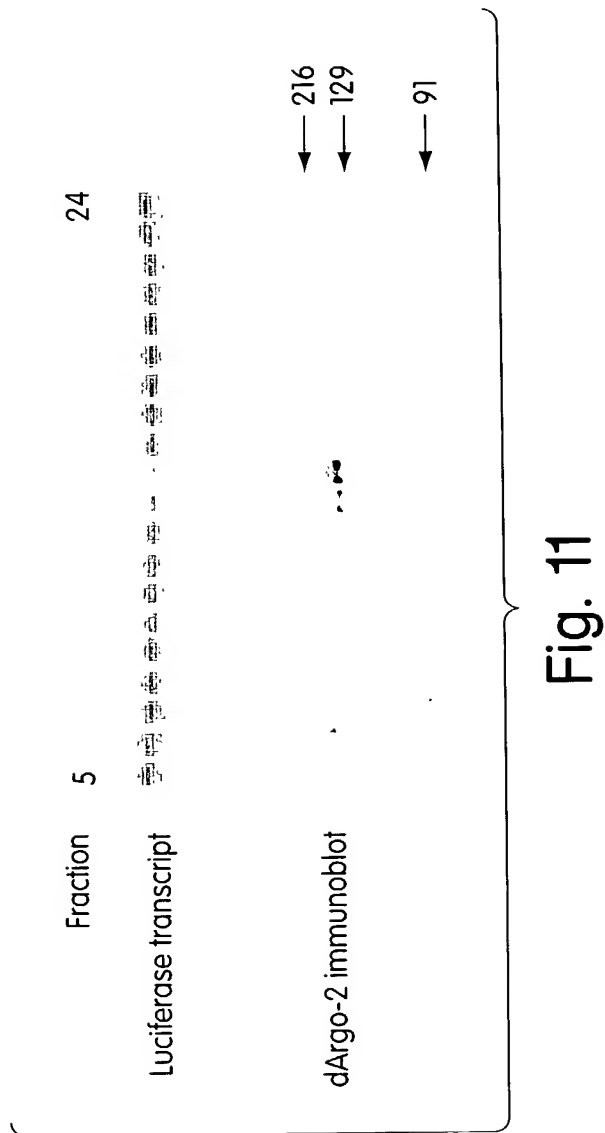


Fig. 10

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Fig. 12

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Fraction 8

Luciferase transcript

dArgo-2 immunoblot

Fig. 13



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At-argonaute-1

Hs-elF2C

Nc-qde-2

Ce-rde-1

Dm-piwi

Dm-sting

Dm-argonaute-1

Dm-argonaute-2

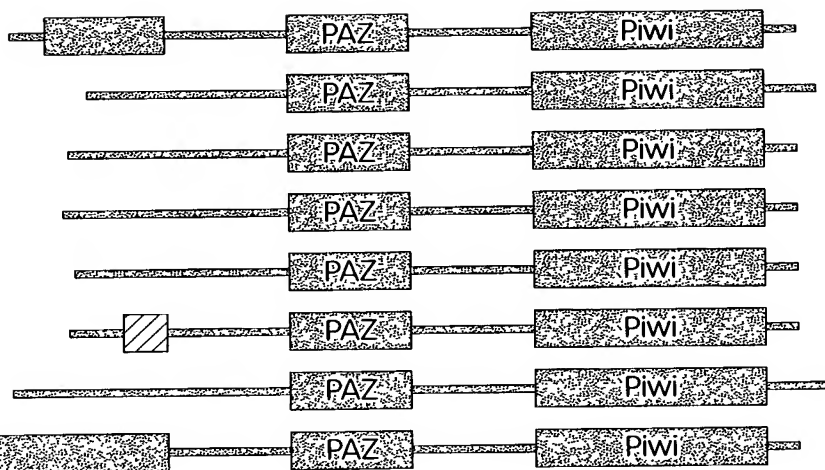


Fig. 14



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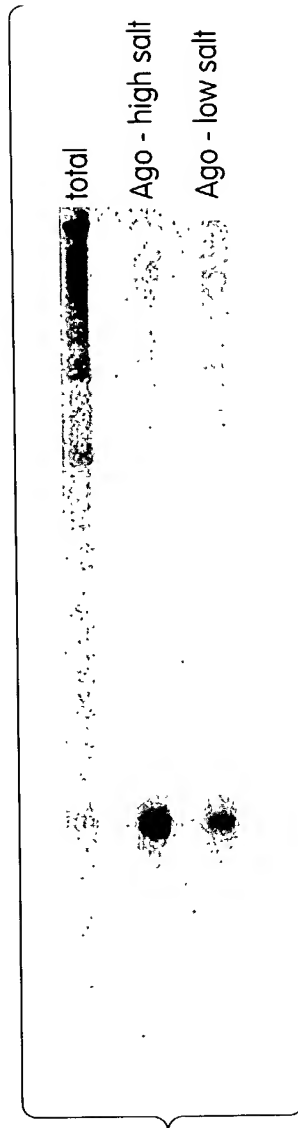


Fig. 15



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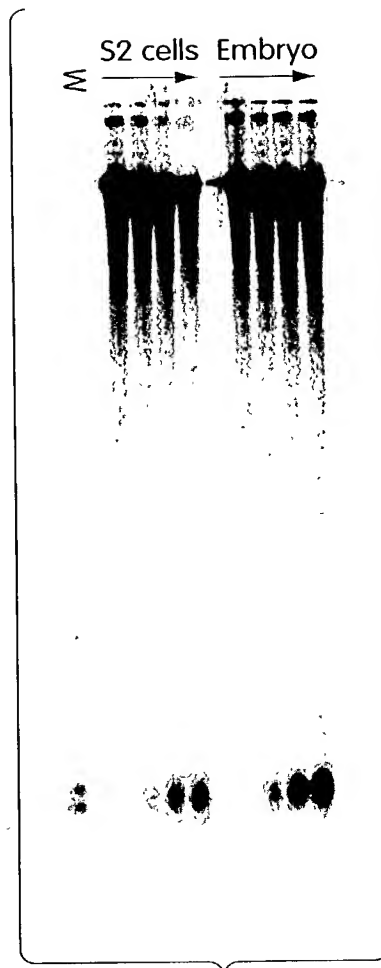


Fig. 16



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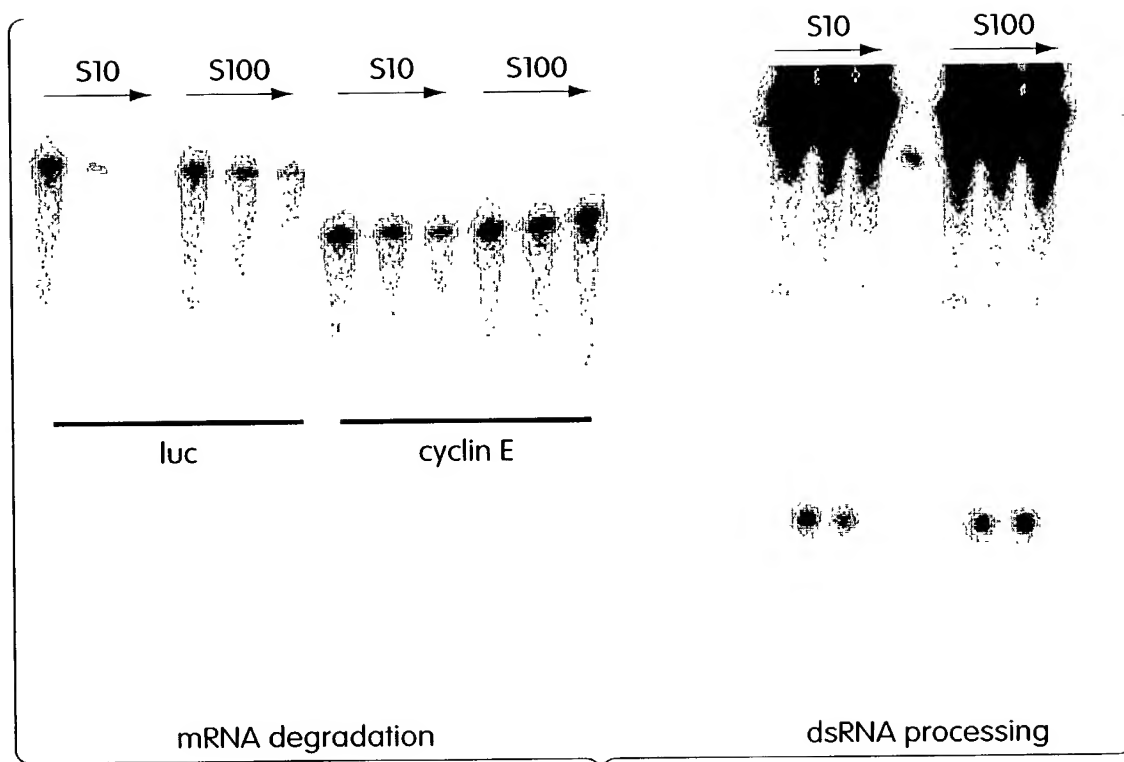


Fig. 17

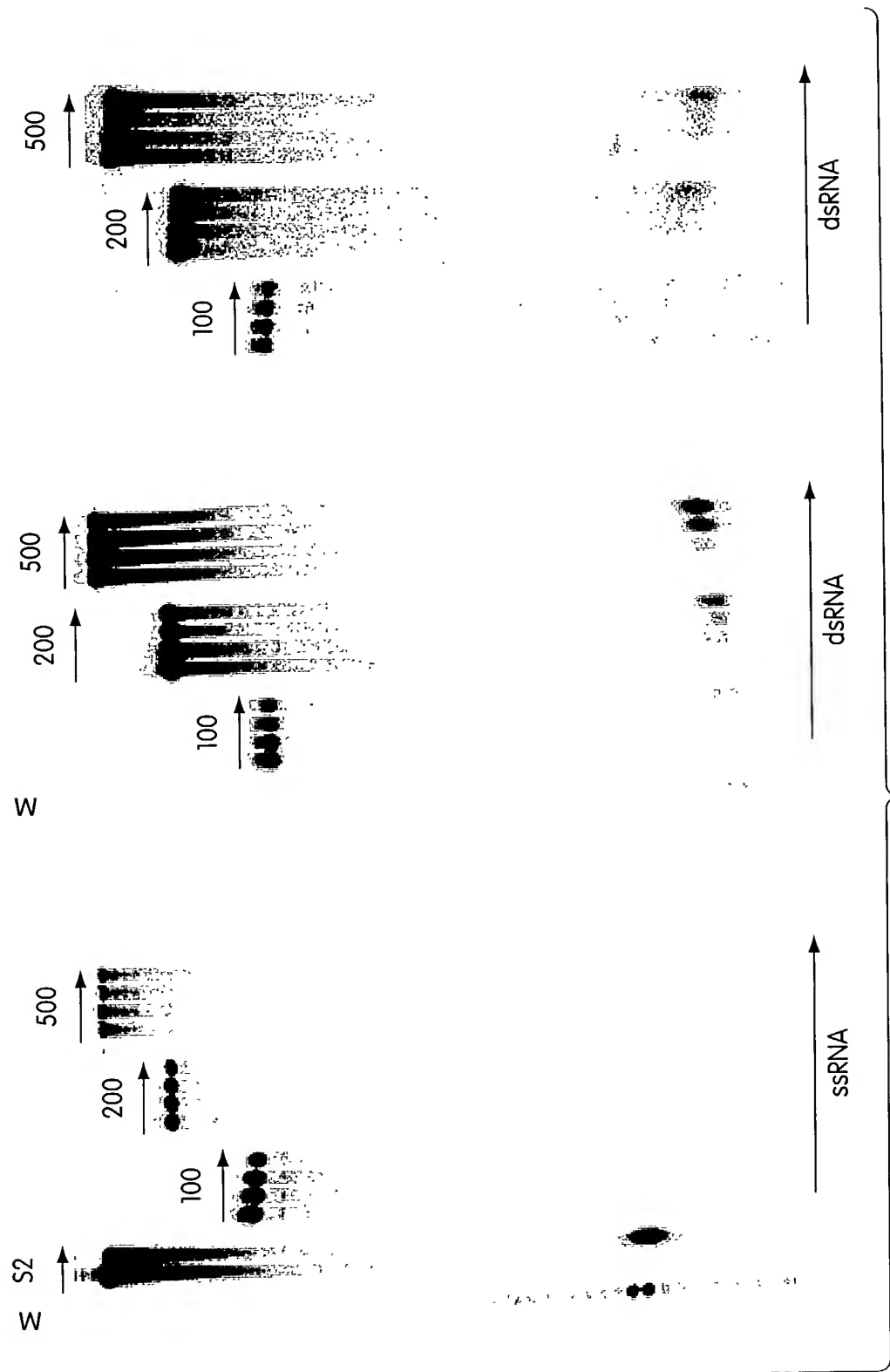


Fig. 18



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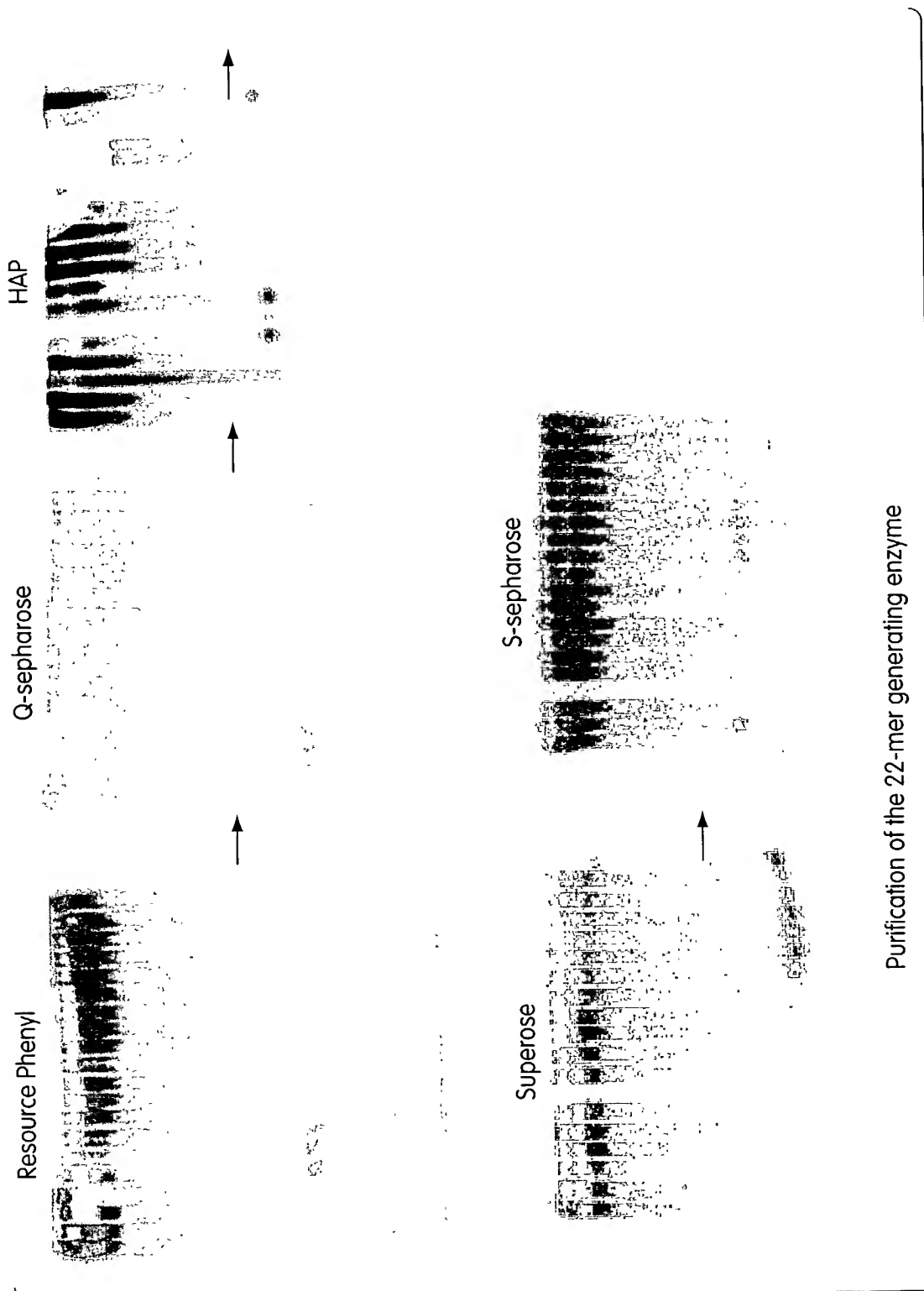


Fig. 19

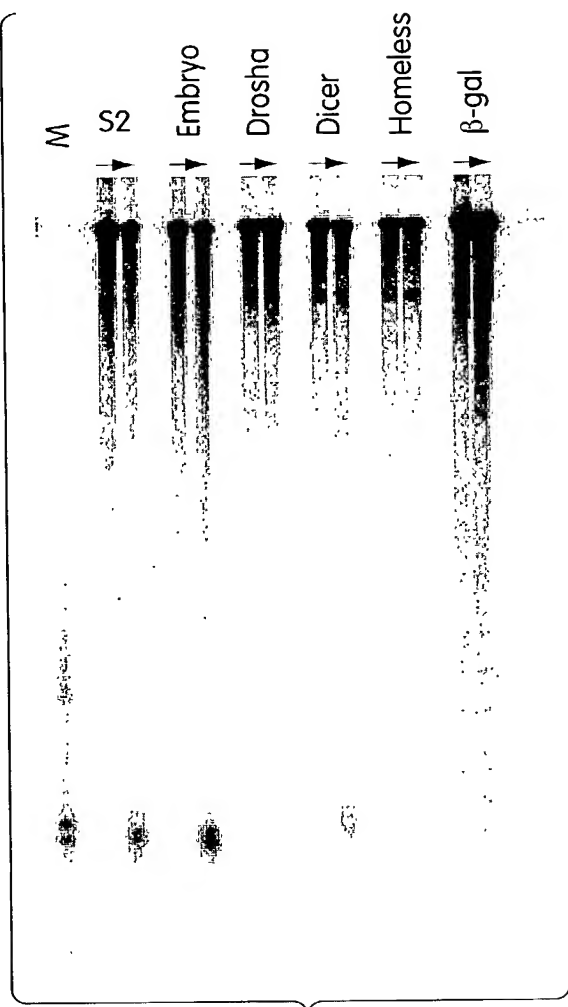


Fig. 20A

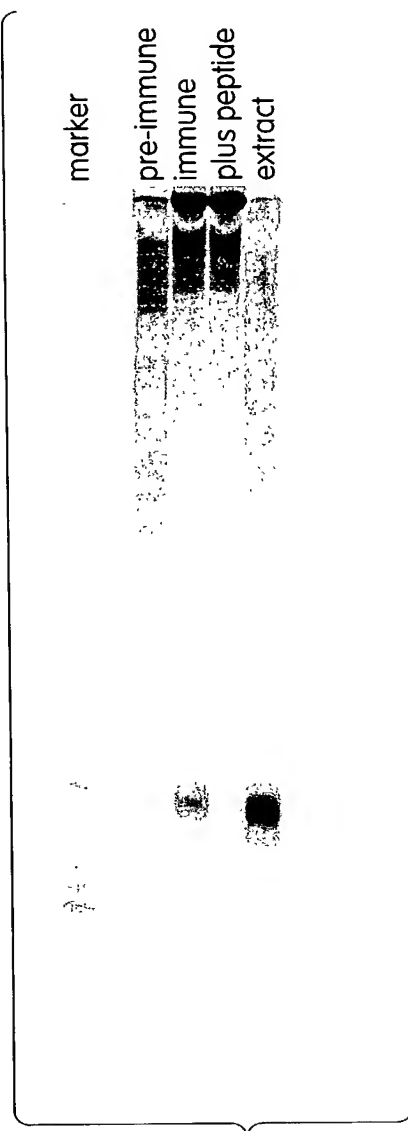


Fig. 20C

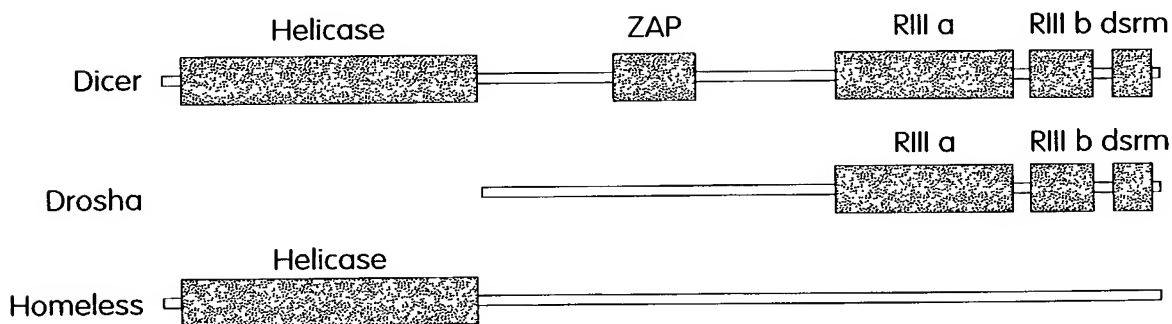


Fig. 20B

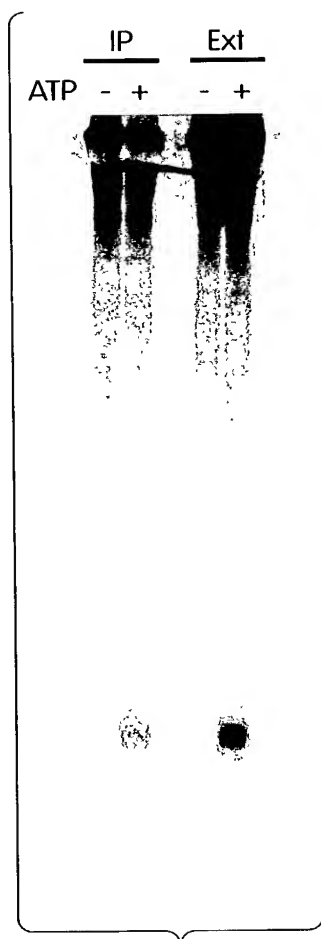


Fig. 21



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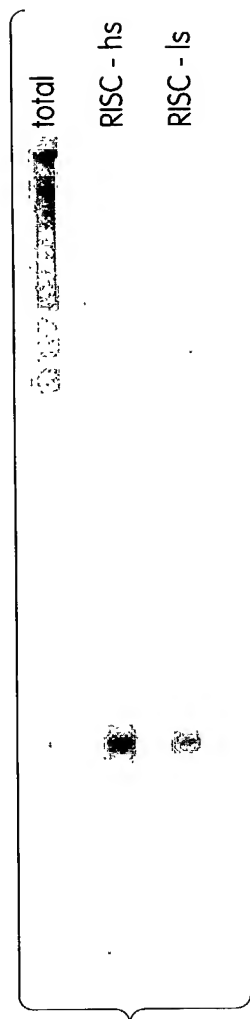


Fig. 22A

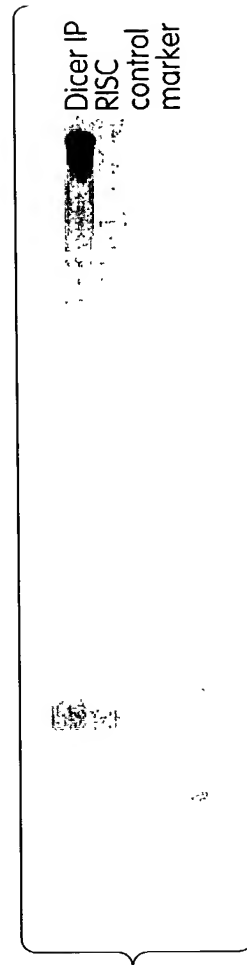


Fig. 22B



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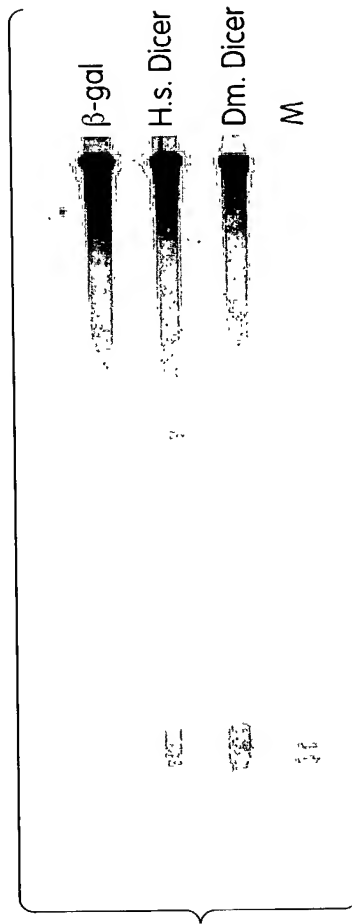


Fig. 23



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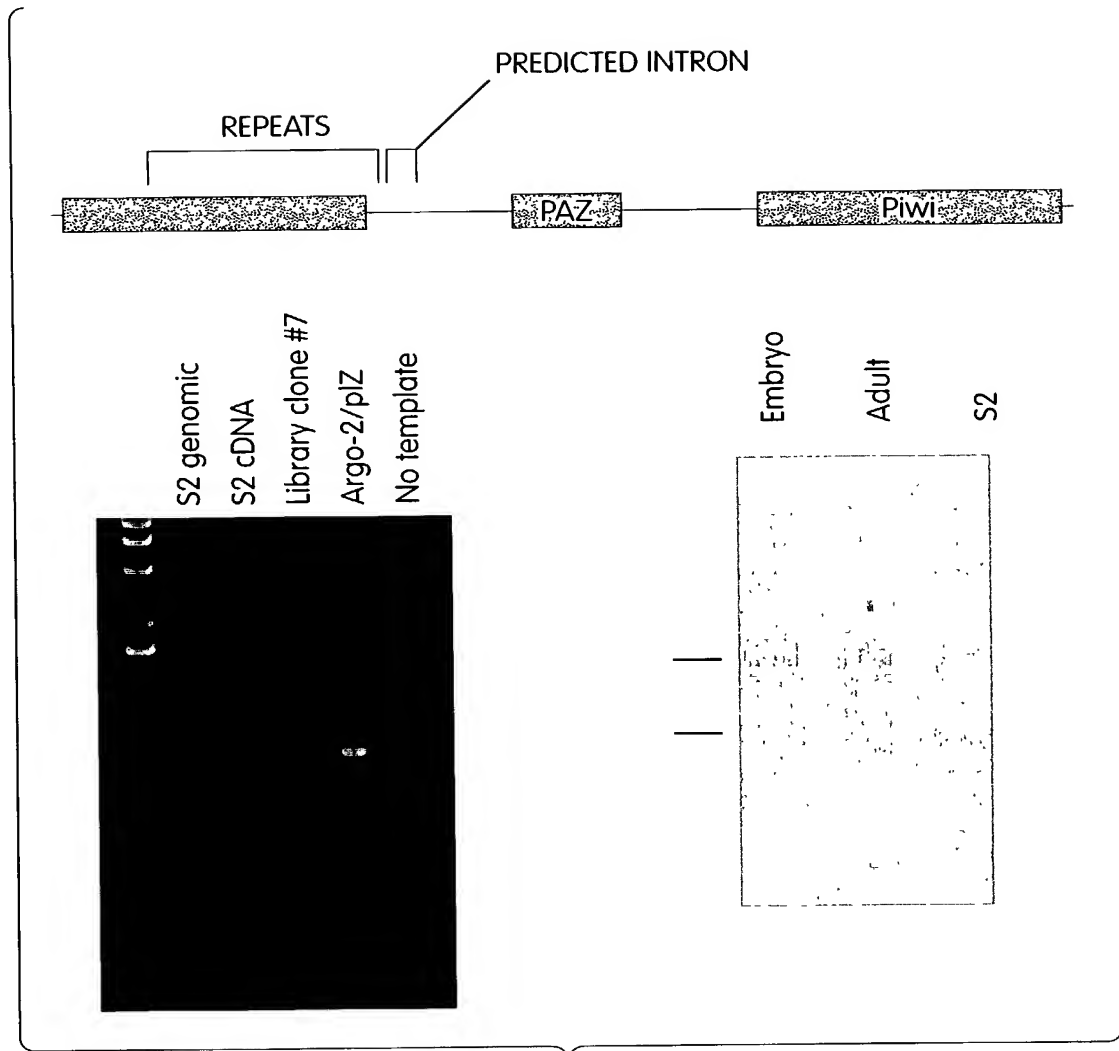


Fig. 25

Fig. 26

Figure 27
Strategy for stable expression of dsRNA in
cultured mammalian cells

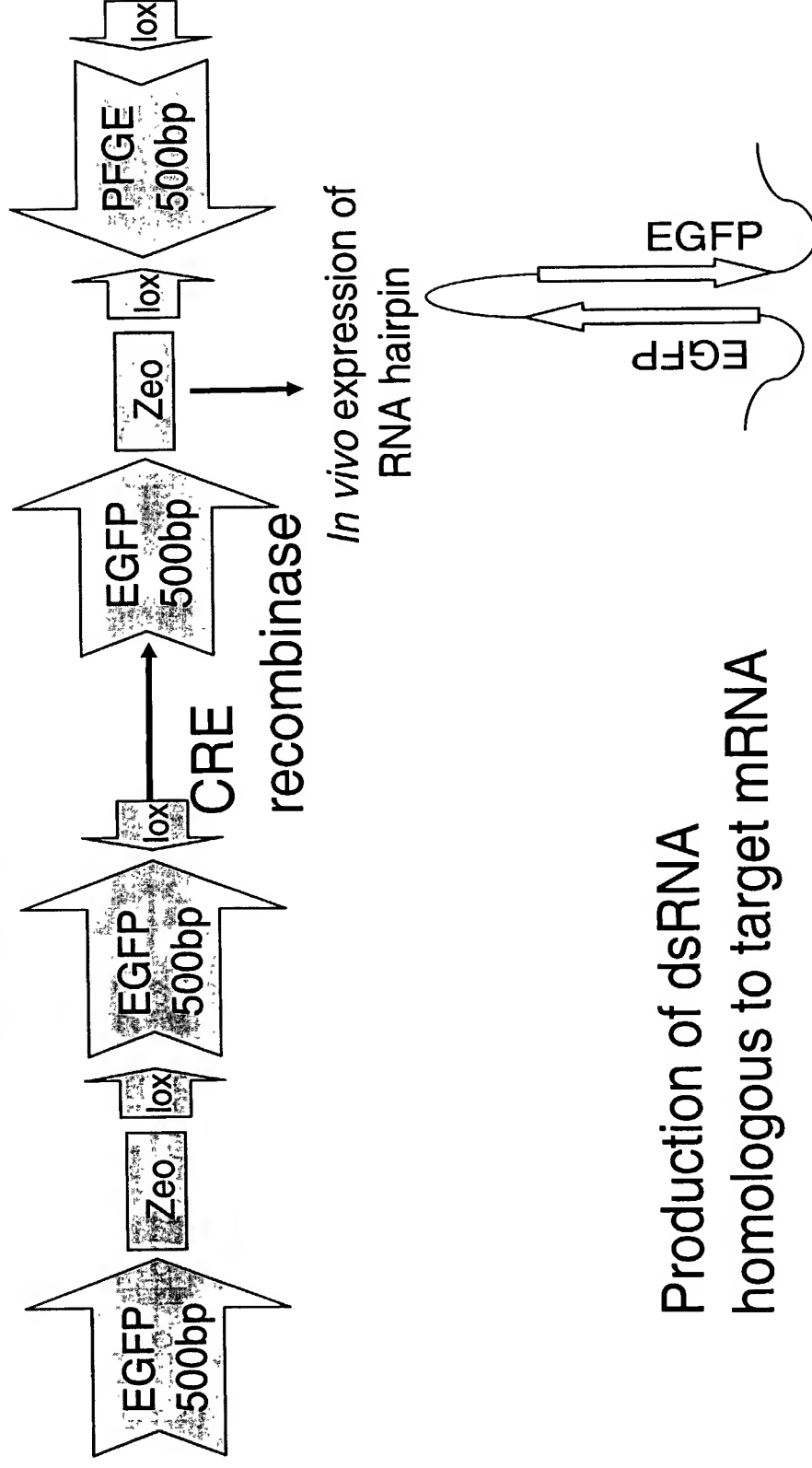
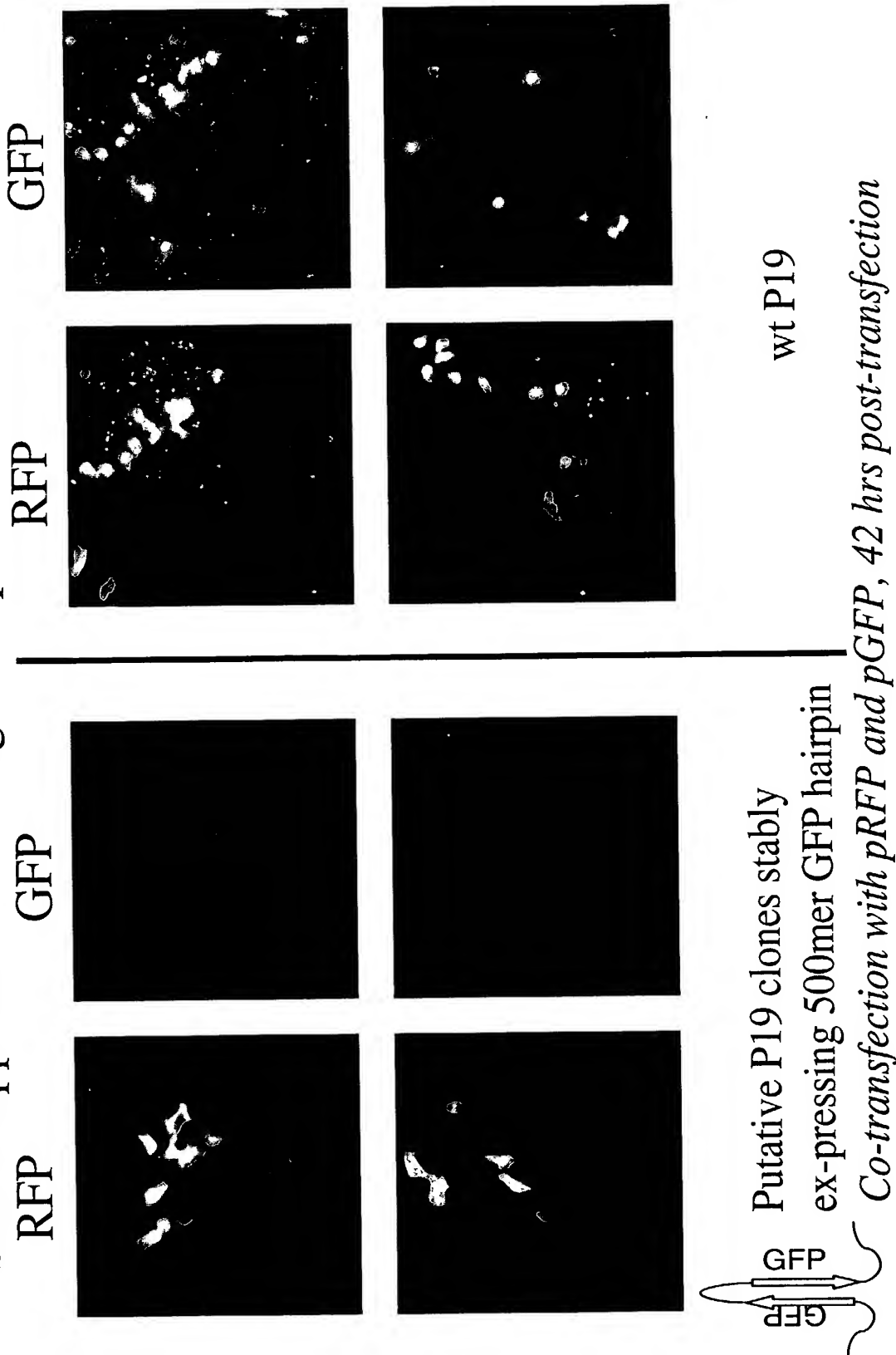




Figure 28
Stable suppression of transgene expression in ammalian cells





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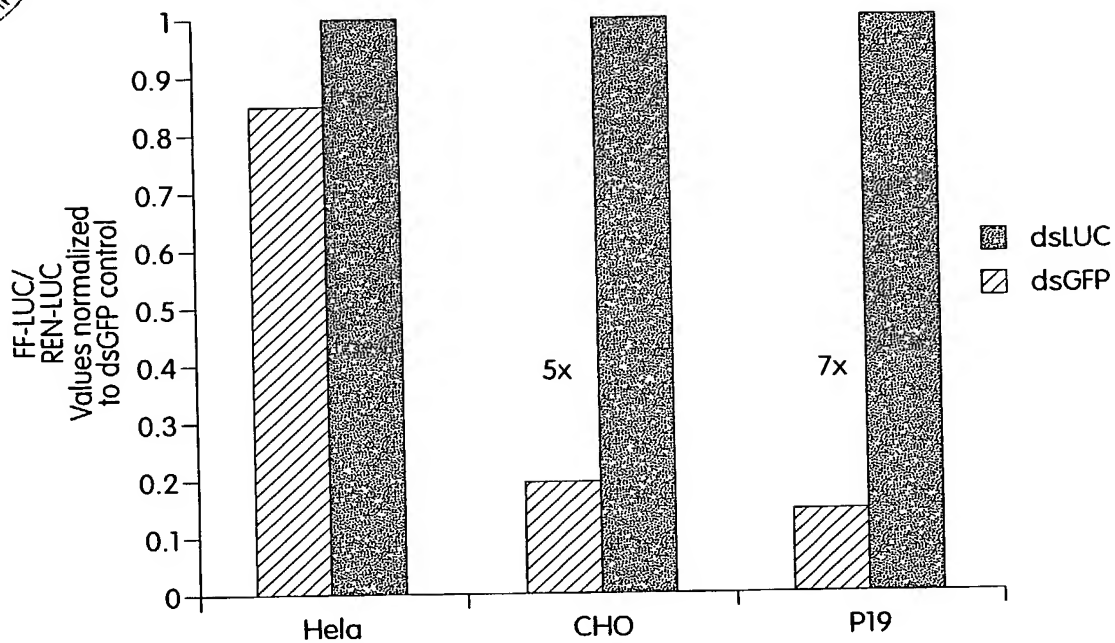


Fig. 29



Figure 30
RNAi in ES cells

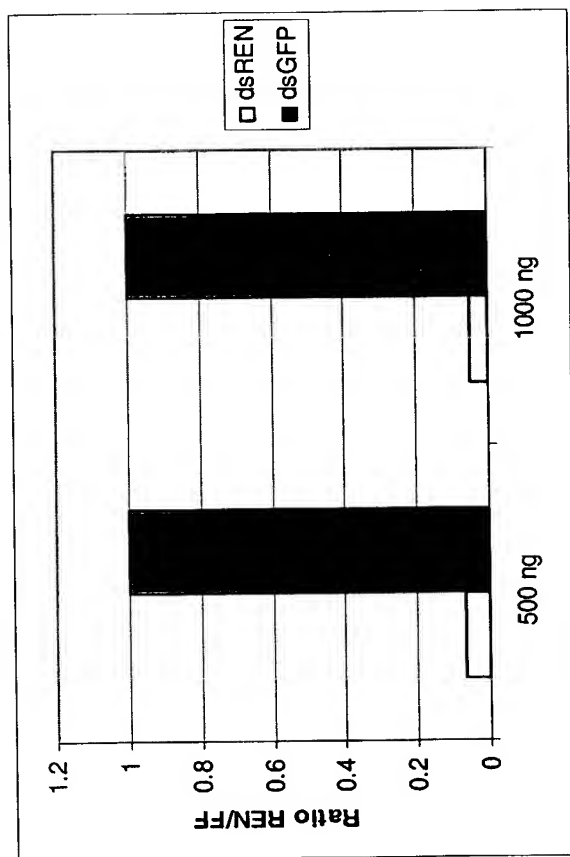




Figure 31
RNAi in mouse embryonic cells (P19)

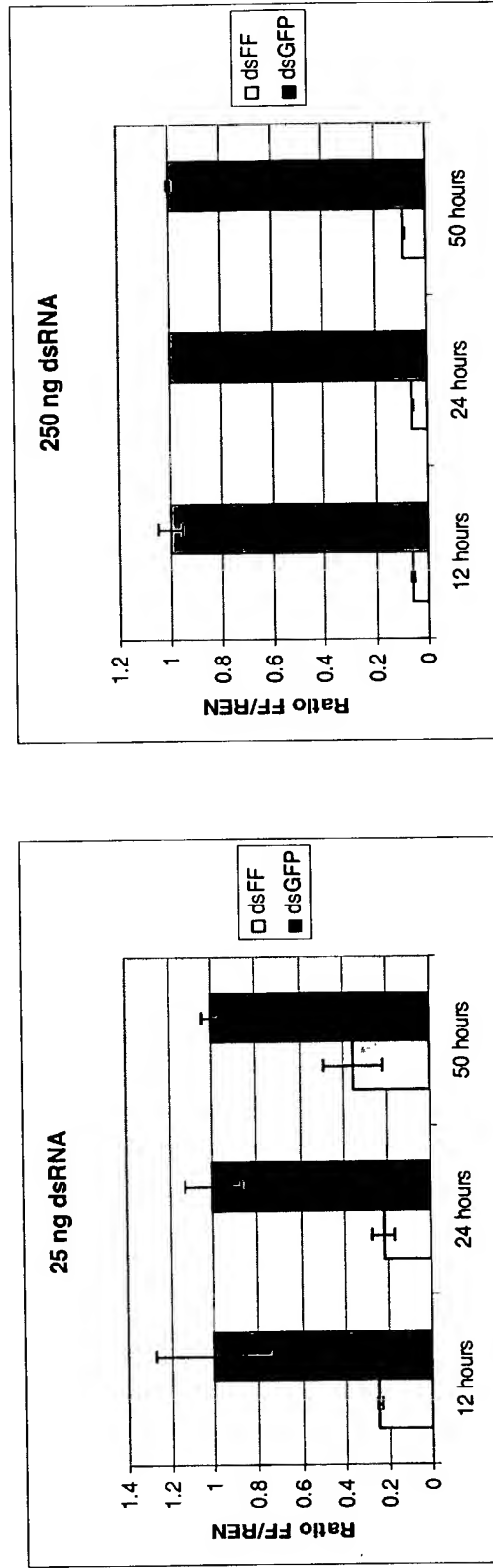




Figure 32
RNAi is post-transcriptional

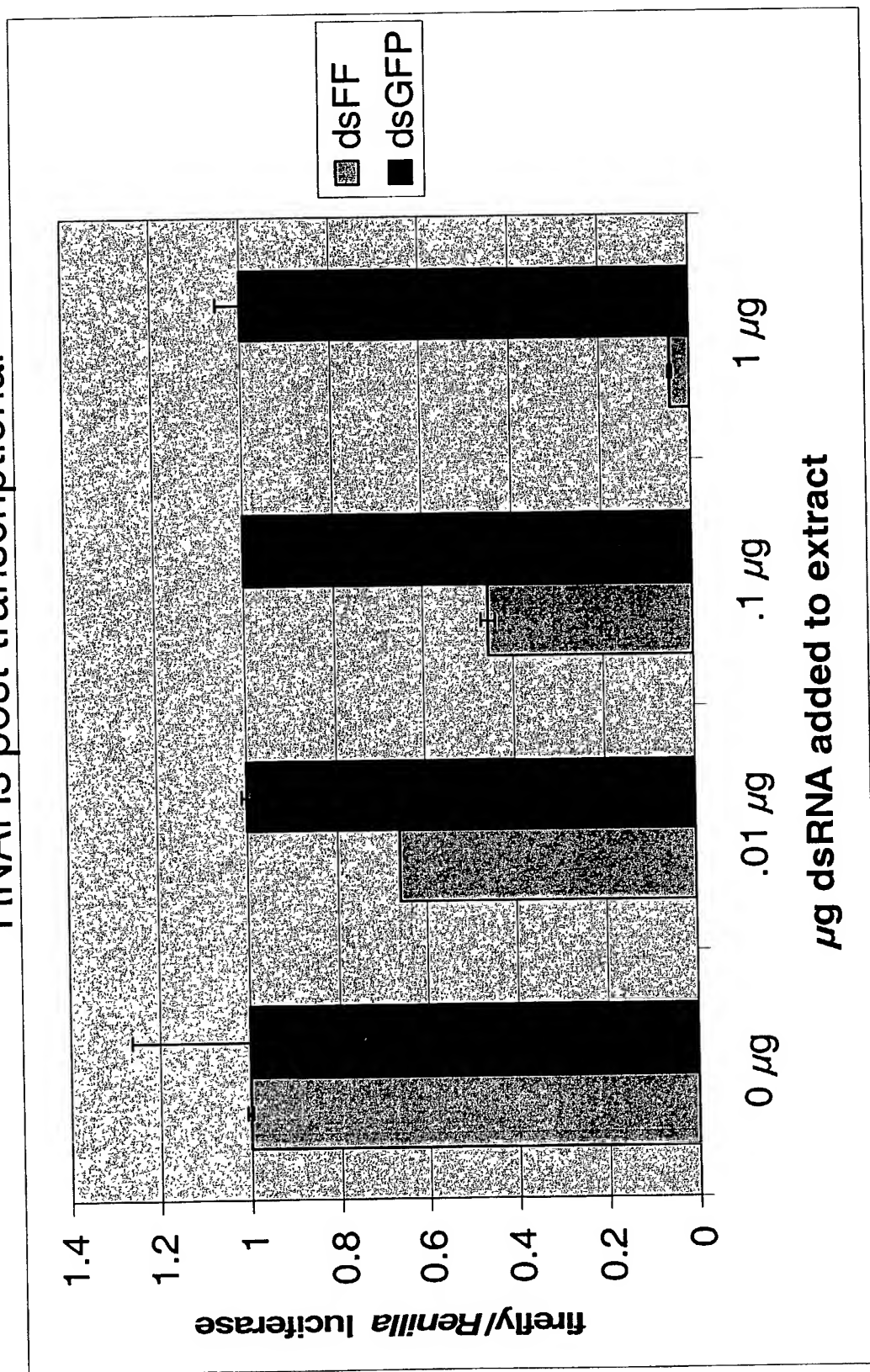
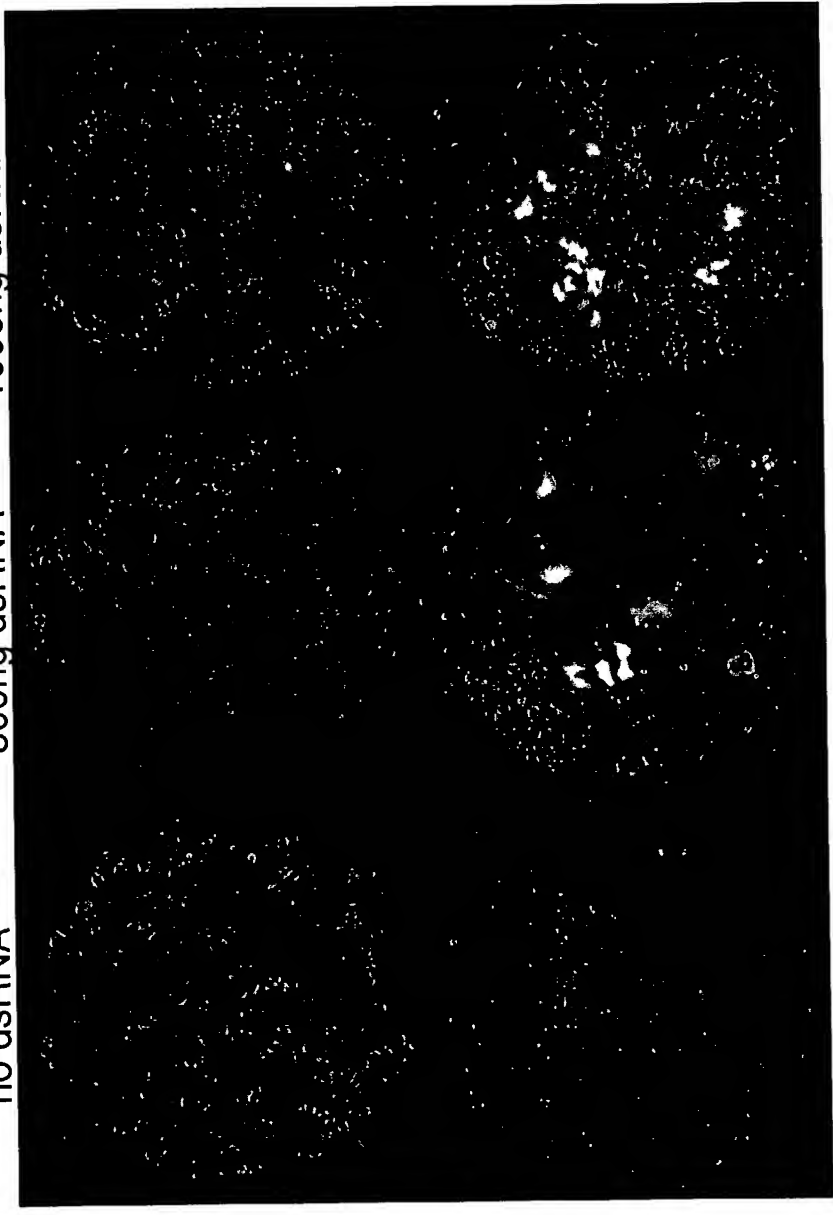




Figure 33

pGFP	+	pGFP	+	pGFP
+	+	+	+	+
no dsRNA	500ng dsRNA	1000ng dsRNA	1000ng dsRNA	1000ng dsRNA



dsFF

dsDicer

P19 GFP hairpin clone number #10
48hrs post-transfection
Fluorescent microscopy superimposed with bright field



Figure 34
Silencing is specific and requires dsRNA

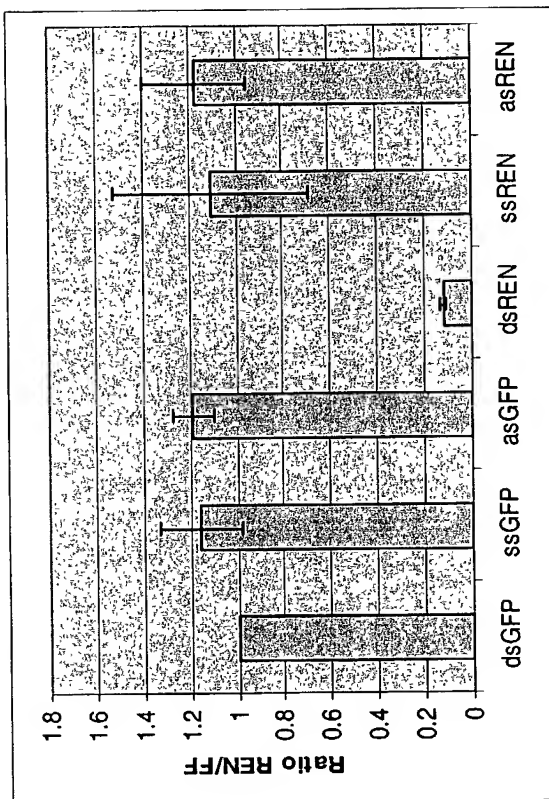
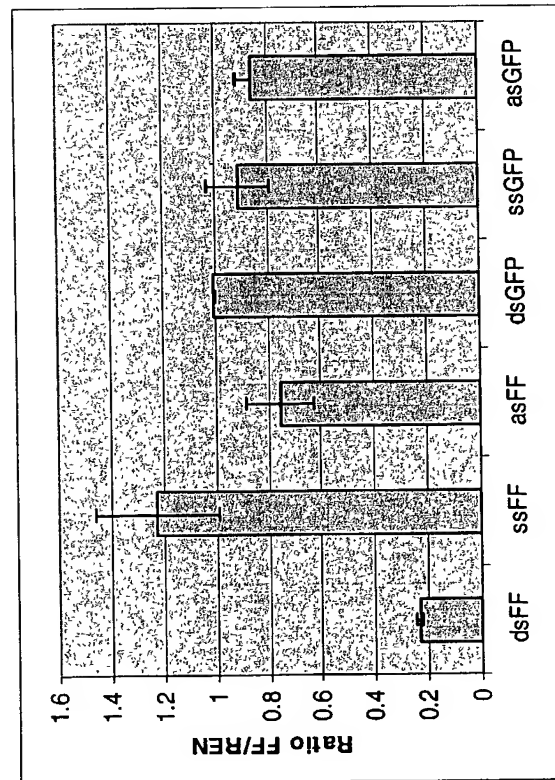
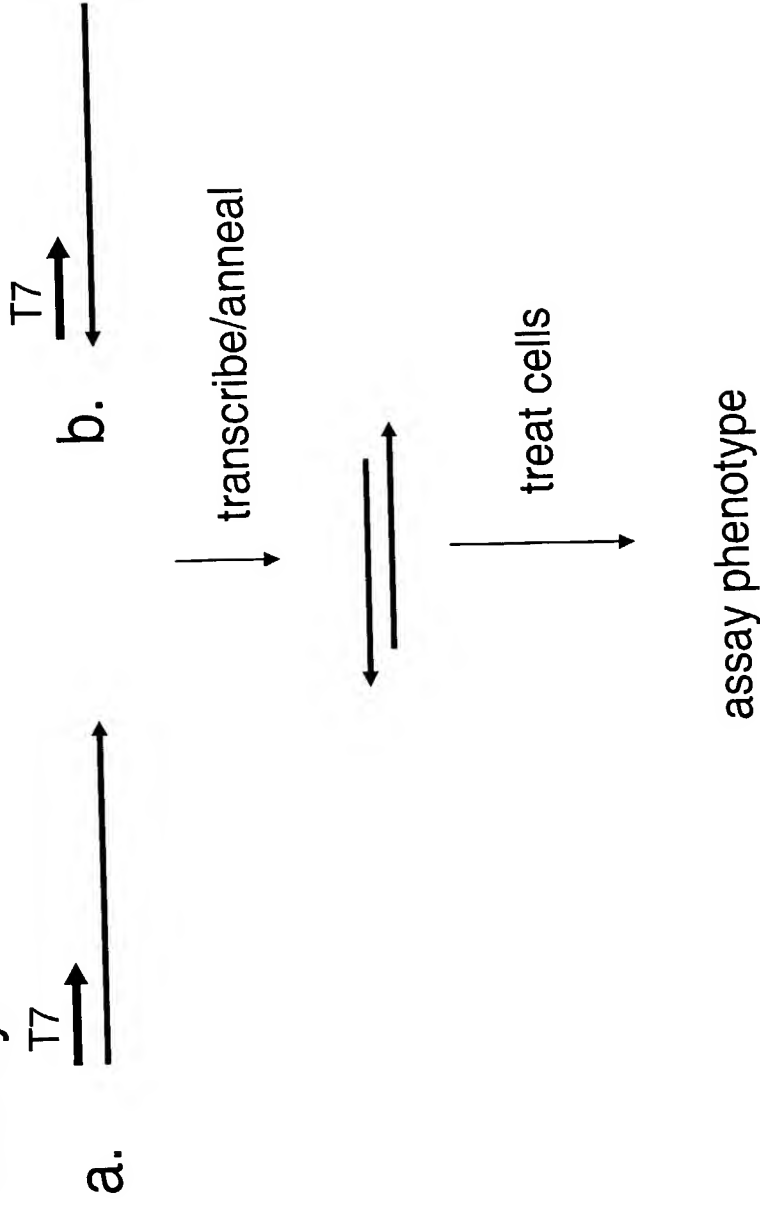




Figure 36
In vitro synthesis of siRNAs by T7 RNA polymerase



DNA synthesis/RNA transcription
~ \$16/siRNA versus ~\$400/siRNA for chemical synthesis
Brings large-scale projects within reasonable budget range



Figure 37

Luciferase siRNA

UAAAGCUUCAUGAGUCGCAUUC
UCGAAGUACUCAGCGUAAGUGA

Luciferase Let-7 like

GAUGGACUGAAAAUUGCGUGGUAUUGGGUUU
GUAGCUGAUUUUAGGGACUAUUAGGUAAA
UAGGGUAUCG
GGGC
UCCCG
C
U

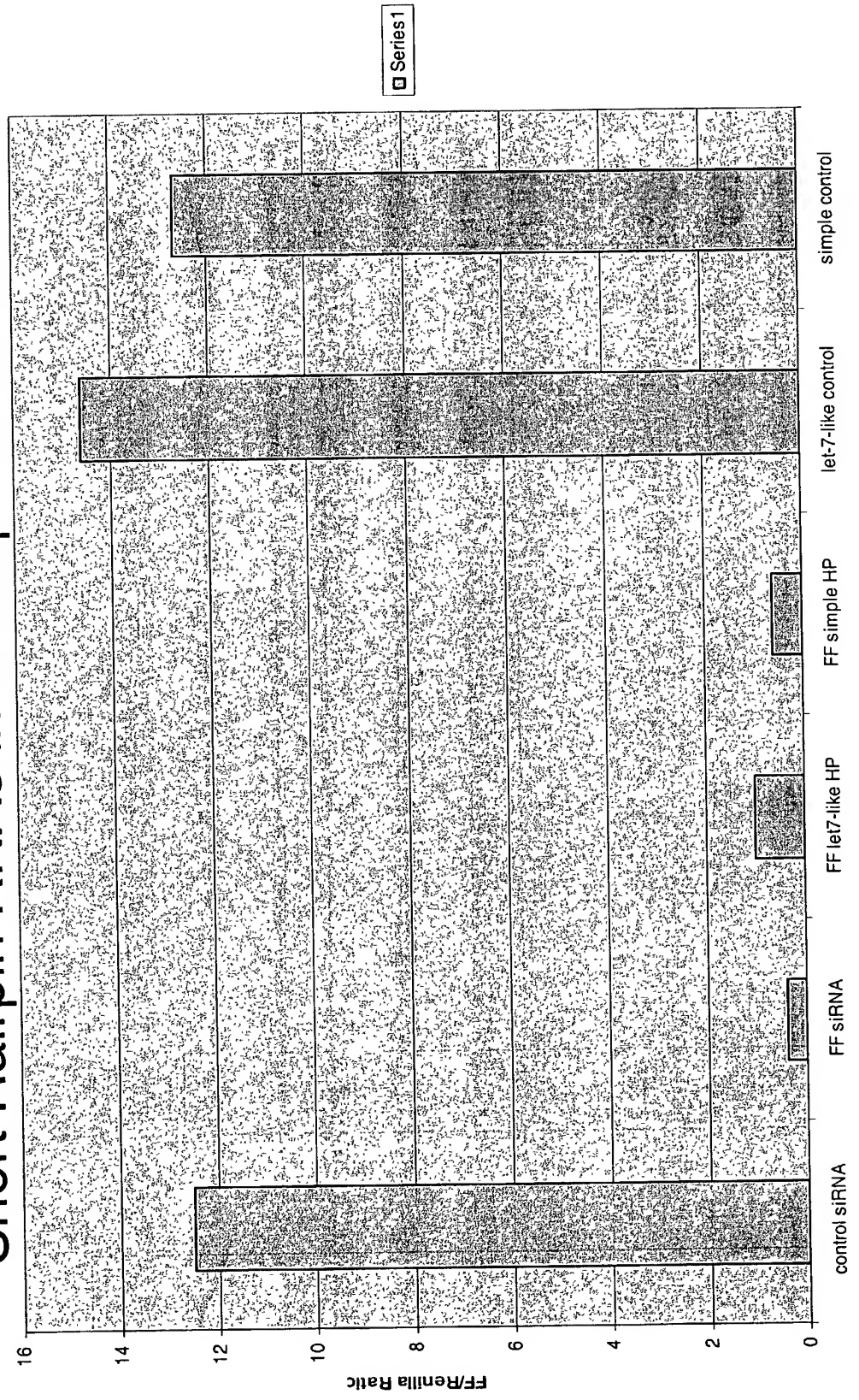
Luciferase simple hairpin

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CAUCGACUGAAAAUCCUGGUAAUCCGUUG
GUAGCUGACUUUAGGGACCAUUAGGCAAC
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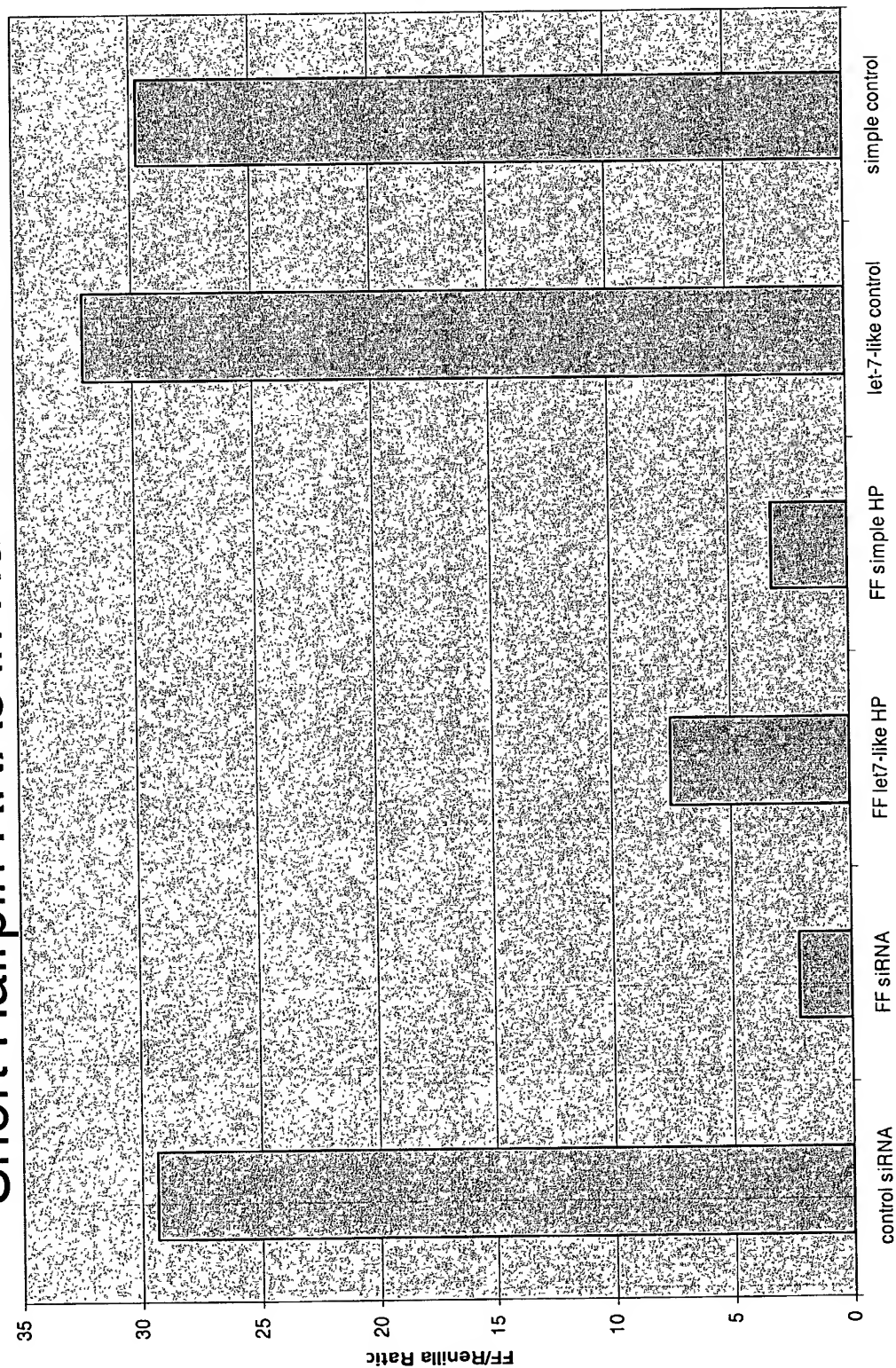
Figure 38
Short Hairpin RNAs in Drosophila S2 cells



11 10 9 8 7 6 5 4 3 2 1

Figure 39

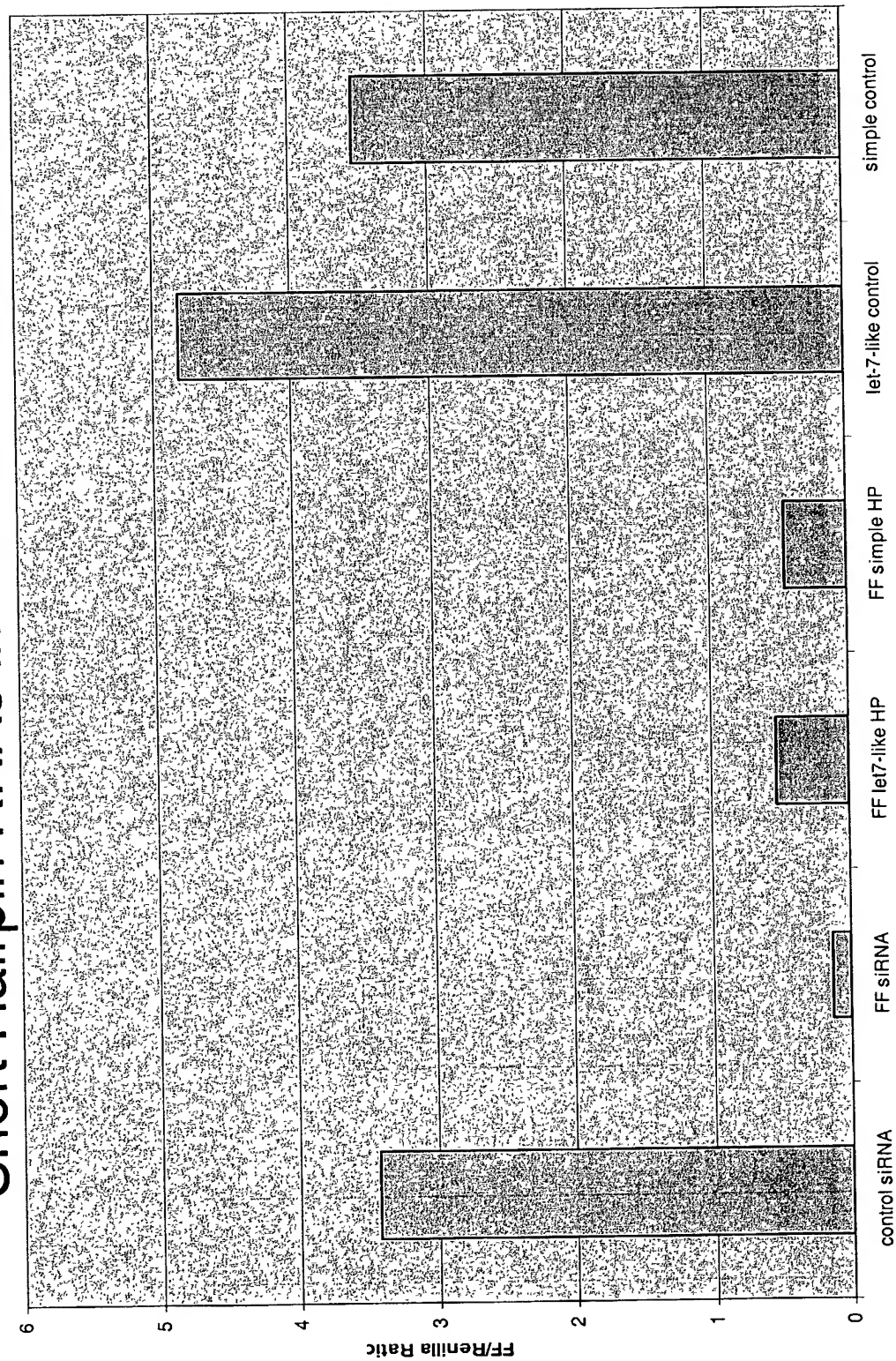
Short Hairpin RNAs in Human 293T cells



11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Figure 40

Short Hairpin RNAs in Human HeLa cells



Series 1



Figure 41
Simultaneous introduction of multiple
hairpins does not produce synergy

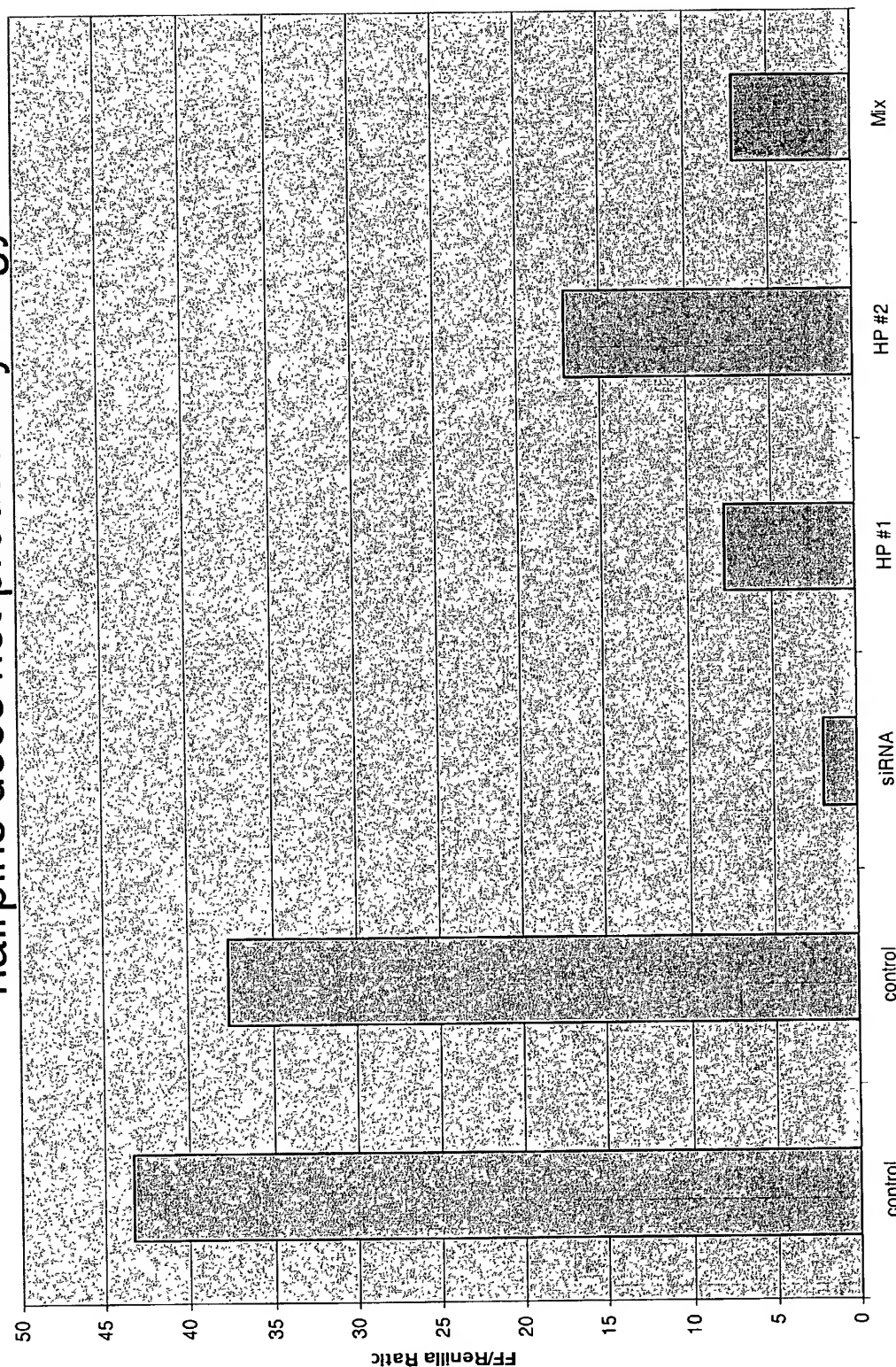


Figure 42
Encoded short hairpins function *in vivo*

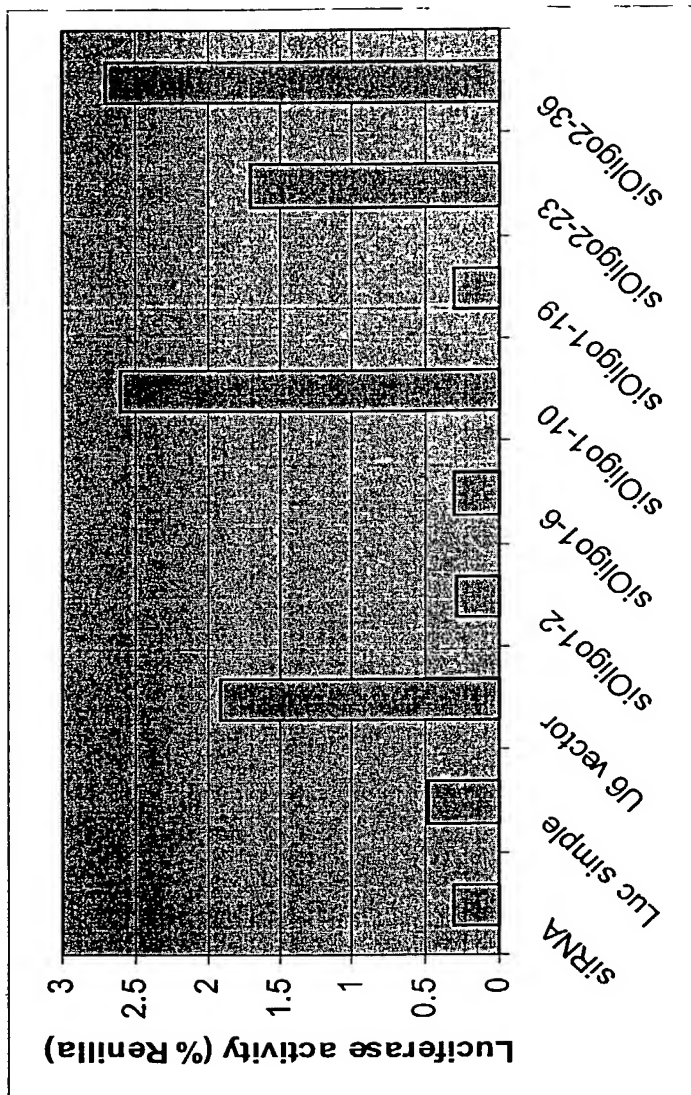
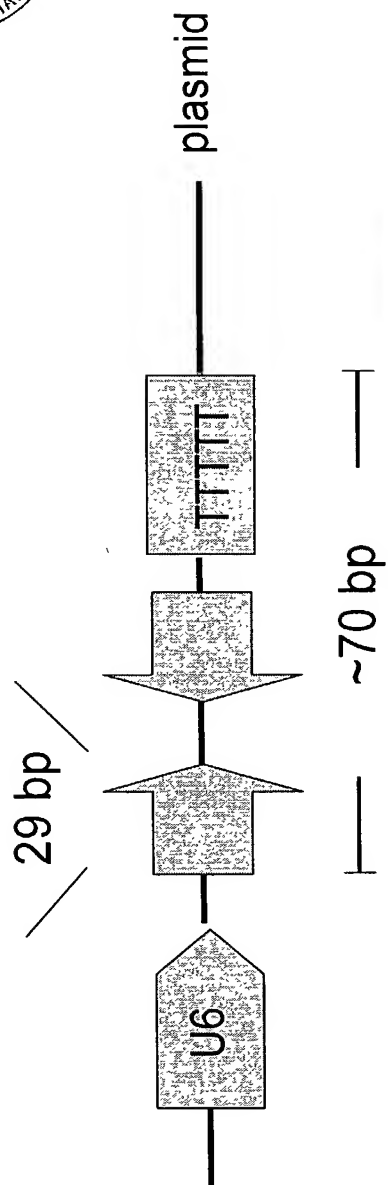
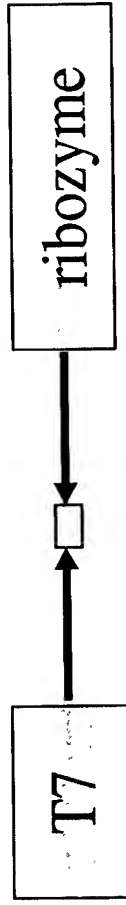
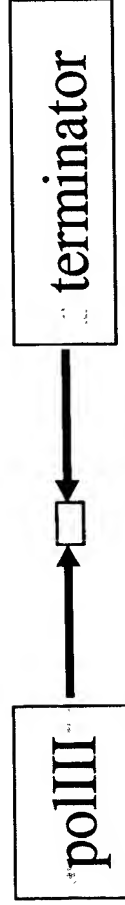


Figure 43

Stable Suppression by short dsRNAs – stable expression strategies

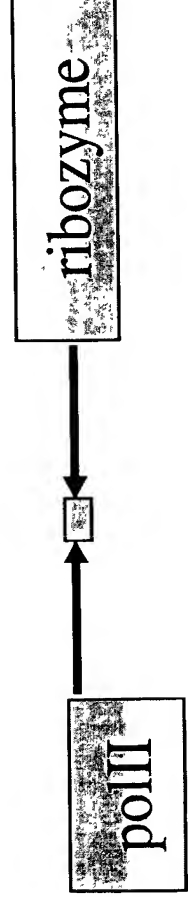


T7 gives site-specific initiation. 3' end formation
Achieved with ribozyme (e.g. hepatitis delta virus ribozyme).



polIII gives site-specific initiation.
Example promoters – U6 snRNA, H1 RNA, SRP RNAs (7SL)
3' end formation
Achieved with native terminator (e.g. TTTT). Leaves the last
TT, so that could be used to pair to transcript.

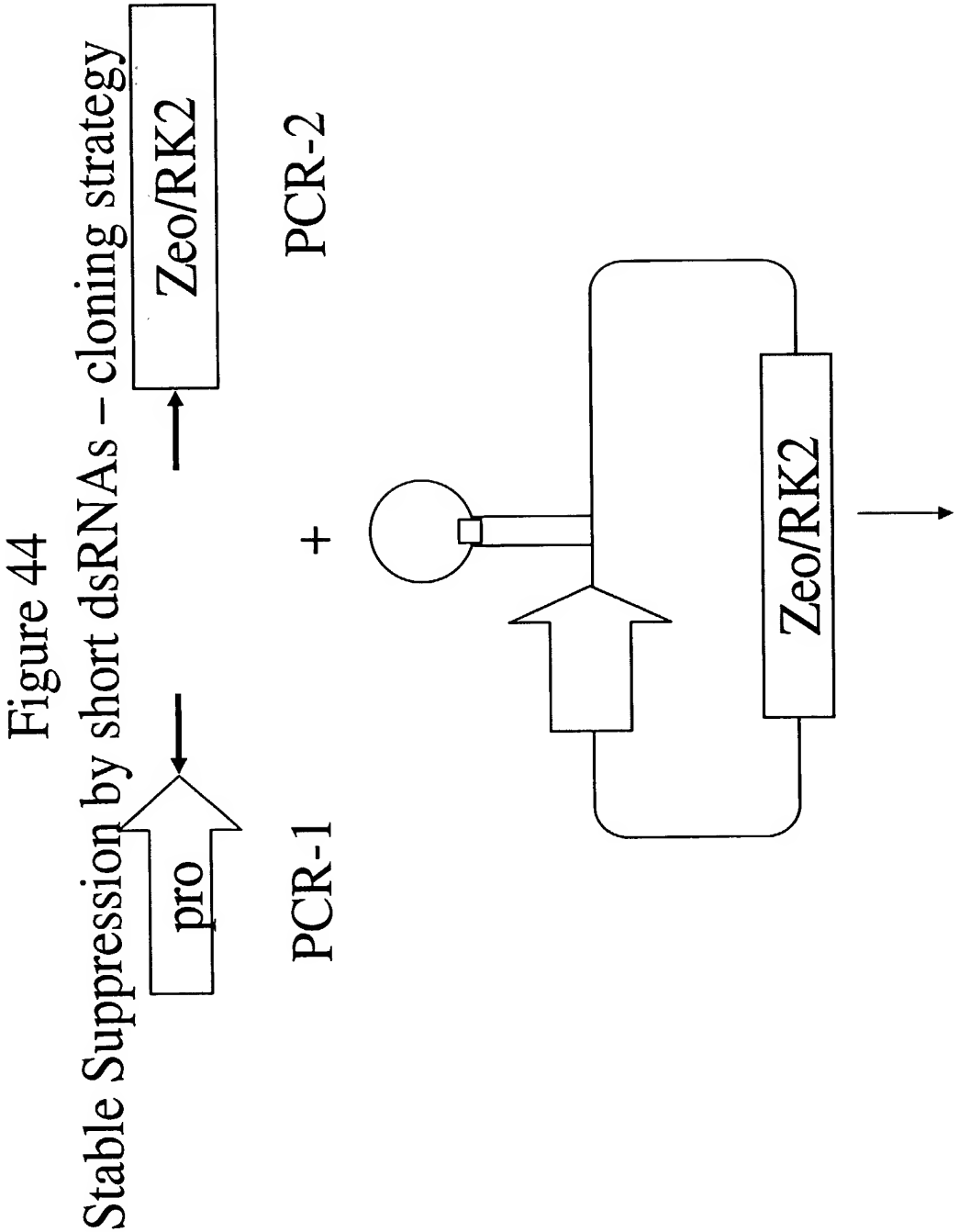
Could also use VA1, tRNA etc but would have to couple with
Ribozyme since those promoters need also internal elements.



polII gives site-specific initiation. Example promoters
Would be U1 snRNA promoters, CMV etc...
3' end formation achieved with ribozyme
(e.g. hepatitis delta virus ribozyme).

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

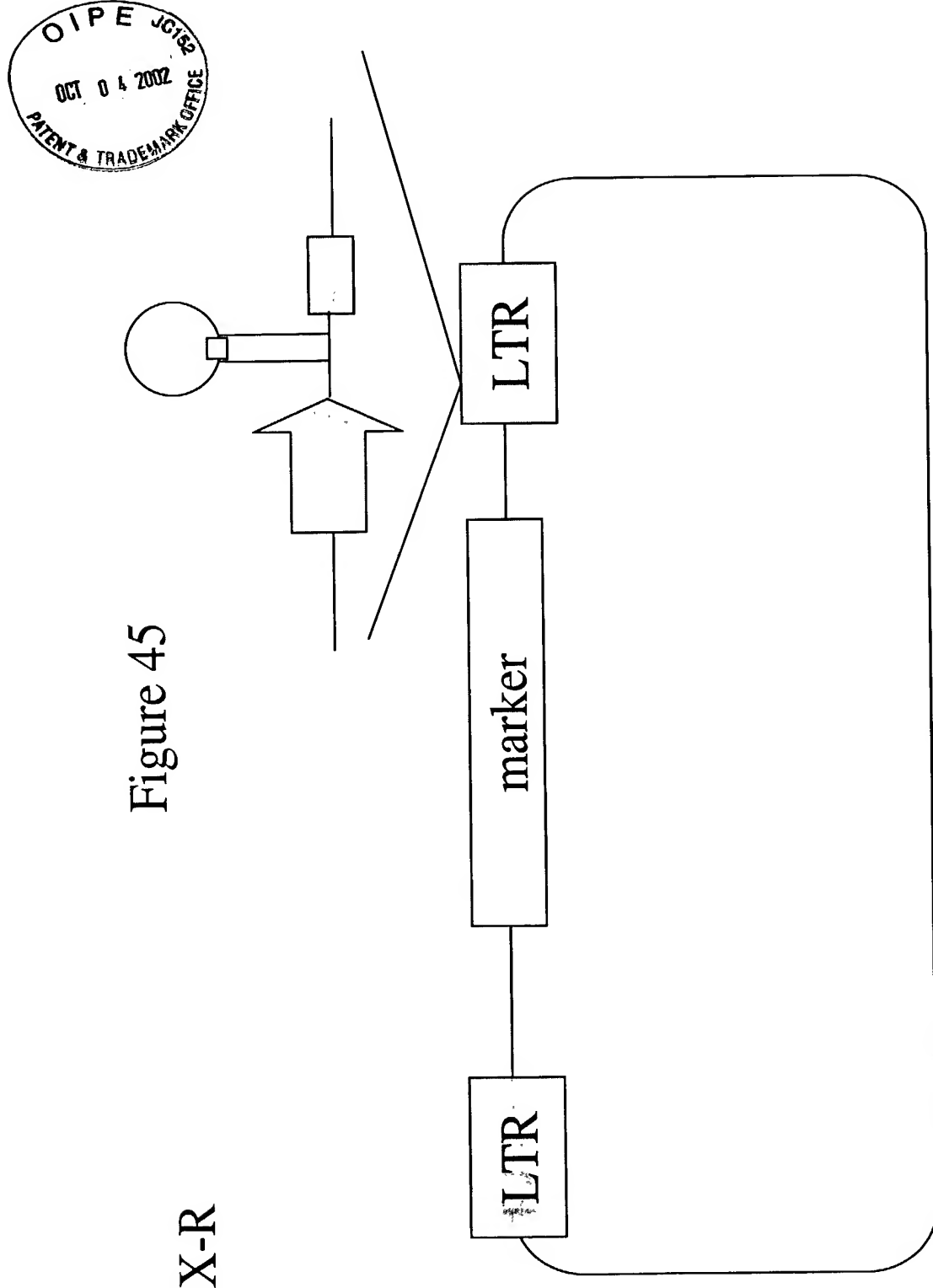




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MaRX-R

Figure 45

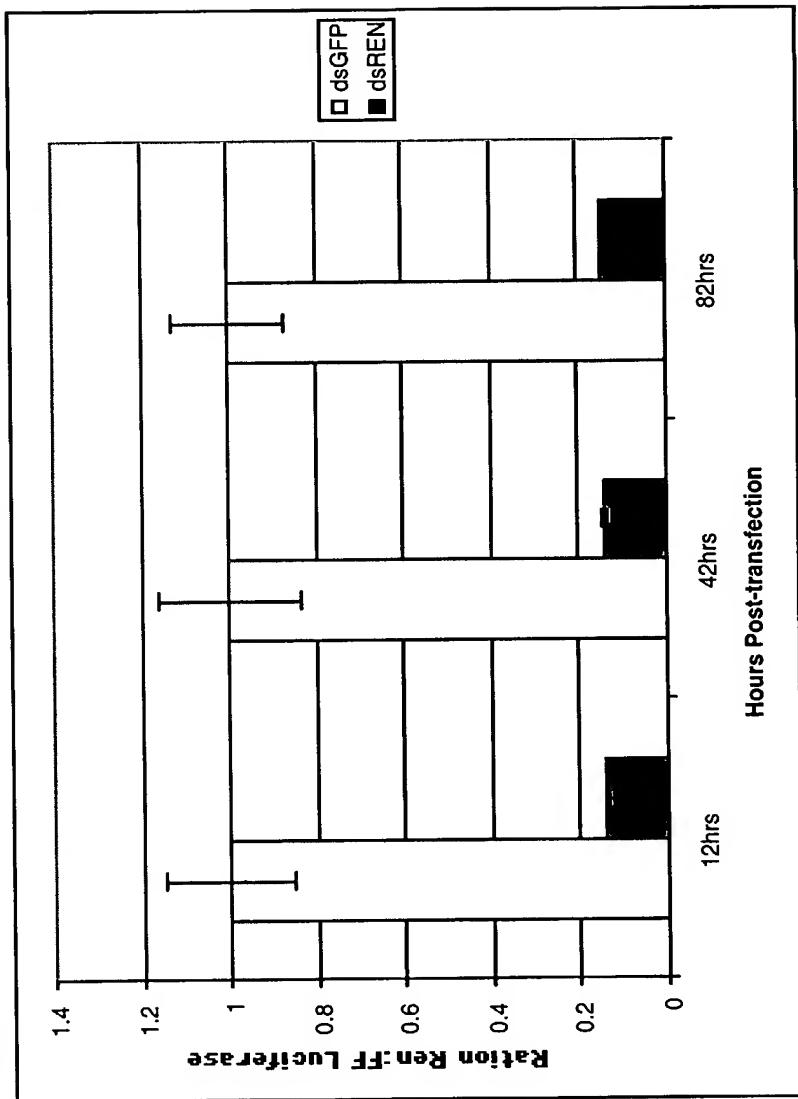


Stable suppression by expressed RNAi



Figure 46

Early Passage PKR $-/-$ MEFs: dual luciferase assay with long dsRNA (~500nt)



12hrs 42hrs 82hrs



Figure 47

Mouse Tyrosinase Promoter

